*Am J Physiol Regul Integr Comp Physiol* 291: R1592–R1601, 2006.
First published August 31, 2006; doi:10.1152/ajpregu.00455.2006.

**CALL FOR PAPERS** | *Sex and Gender Differences in Pain and Inflammation*

# Cross-organ interactions between reproductive, gastrointestinal, and urinary tracts: modulation by estrous stage and involvement of the hypogastric nerve

**Kenneth P. Winnard, Natalia Dmitrieva, and Karen J. Berkley**
*Program in Neuroscience, Florida State University, Tallahassee, Florida*

Submitted 1 July 2006; accepted in final form 26 August 2006

**Winnard, Kenneth P., Natalia Dmitrieva, and Karen J. Berkley.** Cross-organ interactions between reproductive, gastrointestinal, and urinary tracts: modulation by estrous stage and involvement of the hypogastric nerve. *Am J Physiol Regul Integr Comp Physiol* 291: R1592–R1601, 2006. First published August 31, 2006; doi:10.1152/ajpregu.00455.2006.—Central nervous system neurons process information converging from the uterus, colon, and bladder, partly via the hypogastric nerve. This processing is influenced by the estrous cycle, suggesting the existence of an estrous-modifiable central nervous system substrate by which input from one pelvic organ can influence functioning of other pelvic organs. Here, we tested predictions from this hypothesis that acute inflammation of colon, uterine horn, or bladder would produce signs of inflammation in the other uninflamed organs (increase vascular permeability) and that cross-organ effects would vary with estrous and be eliminated by hypogastric neurectomy (HYPX). Under urethane anesthesia, the colon, uterine horn, or bladder of rats in proestrus or metestrus, with or without prior HYPX, was treated with mustard oil or saline. Two hours later, Evans Blue dye extravasation was measured to assess vascular permeability. Extravasation was increased in all inflamed organs, regardless of estrous stage. For rats in proestrus, but not metestrus, either colon or uterine horn inflammation significantly increased extravasation in the uninflamed bladder. Much smaller cross-organ effects were seen in colon and uterine horn. HYPX reduced extravasation in the inflamed colon and inflamed uterine horn, but not the inflamed bladder. HYPX eliminated the colon-to-bladder and uterine horn-to-bladder effects. These results demonstrate that inflaming one pelvic organ can produce estrous-modifiable signs of inflammation in other pelvic organs, particularly bladder, and suggest that the cross-organ effects involve the hypogastric nerve and are at least partly centrally mediated. Such effects could contribute to cooccurrence and cyclicity of distressing pelvic disorders in women.

pelvic pain; inflammation; interstitial cystitis; uterus; colon

NEURAL COORDINATION AND decision-making for many physiological and behavioral functions depends on convergence within the nervous system of information from different bodily regions (for example, see Ref 4). Such is the case, for example, in the control of walking, balance, posture, eye movements, blood pressure, fight/flight responses, and other sensorimotor functions (39). Much of this convergence involves heterotopic interactions between different somatic structures (muscles/skin) or between somatic and visceral structures.

Such coordination is less well understood between different internal organs; that is, viscerovisceral interactions. Studies have shown, however, that neurons in the spinal cord, dorsal column nuclei, solitary nucleus, medullary reticular formation, and thalamus process information that converges from different internal organs located in different functional systems, i.e., the reproductive, gastrointestinal, and urinary tracts (8, 9, 11, 17, 24, 31, 32, 37, 57, 58, 59, 60). In some regions, this processing is robustly influenced by the estrous cycle and hormone manipulations (15, 16).

This cross-system, viscerovisceral convergence supports the concept that the nervous system provides a dynamic substrate [whose characteristics are modified by reproductive status (47)] that permits events occurring in one organ to influence functioning of other organs. Influences like these are clearly important during healthy events, such as parturition, as well as more common functions, like those occurring during coitus, urination, and defecation, which normally are mutually inhibitory (7, 27, 28, 34, 49, 60, 63).

This convergence is likely also significant in pathophysiological situations. It could provide a mechanism by which pathology in one organ influences functioning of another organ. Recent physiological and behavioral data from studies on rodents support such a possibility. Thus acute bladder inflammation in rats increases the motility of the uninflamed uterus (22) and reduces the efficacy of drugs on the uninflamed uterus (21). In addition, acute inflammation of the rat colon or prostate, as well as chronic abdominal pathology (surgically induced endometriosis) produces signs of inflammation in the otherwise healthy bladder (18, 41, 50, 55, 71). Furthermore, the chronic condition of surgically induced endometriosis in rats increases pain behaviors produced by a ureteral stone (26) and induces vaginal hyperalgesia (18). Importantly, the severity of this hyperalgesia correlates with estradiol levels during the rat's estrous cycle (18).

Here, using female rats, we surveyed cross-organ influences between pelvic organs located in three different physiological systems (reproductive-uterine horn, gastrointestinal-colon, and bladder) by inflaming one organ and observing the effect on the other two organs. Because most of the cross-organ effects discussed above support the conclusion that inflammation in one organ indirectly produces signs or symptoms of inflammation in the other organ, we used an outcome measure here that many others have also used to assess inflammation; i.e., extravasation of Evans Blue dye that measures vascular protein permeability (62).

Address for reprint requests and other correspondence: K. J. Berkley, Program in Neuroscience, Eppes Bldg., Copeland St., Florida State Univ., Tallahassee, FL 32306-1270 (e-mail: kberkley@psy.fsu.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Studies have shown that information from the colon, bladder, and uterine horns is conveyed to the central nervous system via the hypogastric nerve (8, 12, 19, 28, 66) and implicate hypogastric nerve involvement in various effects of inflammation in all three organs (for example, see Refs. 13, 22, and 69). Accordingly, we tested the hypothesis that any cross-organ effects observed in this study involved the hypogastric nerve by assessing the effects before and after bilateral hypogastric neurectomy (HYPX).

Furthermore, given earlier findings suggesting that cross-organ effects could be influenced by reproductive status (15, 16, 18), we also tested the hypothesis that any cross-organ effects observed in this study would vary by estrous stage.

## MATERIALS AND METHODS

*Subjects.* Subjects were 116 female Sprague-Dawley rats (Charles River, Kingston, NC), weighing 200–300 g. They were individually housed in wood chip-lined plastic cages, with free access to water and chow, and were maintained on a 12:12-h light-dark cycle with lights on at 0700. Estrous stage was assessed daily by vaginal lavage using the traditional stage nomenclature (5). Only rats that had two complete, regular 4-day estrous cycles before the day of the experiment were used. Measurements were made between ~5 and 8 h after lights on when the rats were in either proestrus (estradiol levels high and progesterone levels low, (5) or metestrus (estradiol and progesterone levels low, (5).

*Experimental protocol.* This protocol was approved by Florida State University's Animal Care and Use Committee, protocol no. 0108.

Rats were anesthetized with urethane (1.2 g/kg ip) and placed on a heating pad to maintain body temperature at ~37°C. Anesthetic level was monitored by periodic tests of corneal and foot-pinch reflexes. Supplemental doses of anesthetic were rarely needed. The jugular vein was catheterized, and a midline abdominal incision was made to expose the pelvic cavity. As described in detail below, either the colon, right uterine horn, or bladder was treated for ~2 h with saline or 10% mustard oil (MO) dissolved in mineral oil before death. A 2-h treatment duration was chosen to be consistent with the duration of inflammation used by other studies in which the outcome measure was either remote protein extravasation (PE) or effects on central neuronal activation (6, 74, 75).

In subgroups of rats whose colon, uterine horn, or bladder was to be injected with MO, HYPX was performed ~60 min before treatment. The HYPX was done by identifying the left and right hypogastric nerves where they exit distally from the inferior mesenteric ganglion (IMG), freeing them completely from connective tissue, cutting them, and separating the cut ends from each other. The 1-h delay was chosen because other studies have shown that the effects of HYPX are evident at that time (22).

Evans blue dye was injected via the jugular vein (50 mg/kg in saline) 2 h after organ treatment. Approximately 30 min later, the dye was flushed out of the vascular system by transcardial perfusion with saline. Immediately thereafter, samples of the right and left uterine horn, the colon, and the bladder were each harvested identically in all groups, as follows. The uterine and colon samples were 1-cm-long segments cut out from the region that had been (or would have been) treated with the MO or saline solutions. The entire bladder was removed at the bladder-urethral junction. All samples were bisected longitudinally, rinsed with saline, patted dry, and weighed.

The harvested samples were placed in individual vials containing 2 ml of formamide (Sigma, St. Louis, MO) to extract the dye (62). Samples were stored at ~50°C for 24 h. The optical density of the formamide extraction solution was measured spectrophotometrically (wavelength: 620 nm, UV: 1601; Shimadzu, Columbia, MD). A calibration curve was used to calculate the amount of dye extracted from the tissue (i.e., μg EB/g tissue). This measurement unit is hereafter referred to by its common acronym PE.

*Experimental groups.* PE values were measured in samples of the right uterine horn, colon, and bladder taken from 18 groups of rats ($n$ = 6 or 7/group) as follows: *Group 1*: colon treated with MO, studied in proestrus; *Group 2*: colon treated with MO and HYPX, studied in proestrus; *Group 3*: colon treated with saline, studied in proestrus; *Groups 4–6: colon treatments as in* groups 1–3*, but studied in metestrus;* Groups 7–12*: uterine horn treated as in* groups 1–6*, but studied in metestrus;* Groups 13–18*: bladder treated as in* groups 1–6 *or* 7–12*, but studied in metestrus.*

At the end of the study, an additional group was run to control for the possibility that the additional 1-h delay before the administration of MO in the HYPX groups made any difference in the results. In this extra control group, a sham HYPX surgery was carried out in rats in proestrus ($n$ = 4) in a manner identical to HYPX but without cutting the two nerves. This sham surgery was followed 1 h later by inflaming the colon with MO. The effects in this group did not differ significantly from those in the experimental group in proestrus treated only with MO.

*Treatment of the colon.* A clean, dry pad was placed under the hindquarters to raise the caudal end of the rat's body ~1° from horizontal. A catheter (outer diameter, 2.0 mm) was inserted transrectally and gently pushed rostrally ~60 mm to reach the descending colon at a level ~1 cm rostral to the bladder and at approximately the same rostrocaudal level as the middle of the uterine horns. Next, 0.5 ml of either MO or saline was infused into the colon, and the catheter was removed. Any leakage of the MO solution (which is pale yellow) that was infused into the colon could be identified by monitoring the dry pad underneath the rat. No leakage was observed in any of the rats used for analysis.

*Treatment of the uterine horn.* The caudal end of the right uterine horn was tightly ligated with 4.0 silk suture, taking care not to injure any neighboring structures. This ligation prevented the solution that was infused into the horn from leaking out of the cervix into the vagina but produced no gross signs of uterine ischemia. Next, ~0.25 ml of MO or saline was injected into the rostral part of the right uterine horn via a 22-gauge needle. The needle was then removed, and, to prevent leakage, the small injection site was immediately clamped with a small hemostat. A gauze pad that had been placed under the injection area of the uterine horn was monitored to identify any leakage of the solution. No leakage was observed in any of the rats used for analysis.

*Treatment of the bladder.* The bladder was catheterized transurethrally (outer diameter, 1.1 mm), placing the tip of the catheter in the middle of the bladder lumen without touching its walls. A silk suture was tied around the skin over the urethra to secure the catheter's position and prevent leakage. Then, after emptying the bladder by very gentle suction through the catheter (using filter paper in the catheter) and gentle pressure on the sides of the bladder using cotton-tipped applicators moistened with warm saline, 0.5 ml of either MO or saline was infused into the bladder and left in place for ~2 h. A gauze pad that had been placed under the rats was monitored to identify leakage. No leakage was observed in any of the rats used for analysis.

*Data analysis.* Significance was assessed using SPSS version 12 or 13 software. Repeated-measures ANOVAs were used to determine significance of the effects of treatment (MO, MO+HYPX, saline; between subjects) and effects on different organs (colon, uterus, bladder; within subjects). If significant, one-way ANOVAs were used to assess the significance of differences in the effect of treatment groups for each tissue treated (i.e., colon, uterus, bladder), followed, if significant, by post hoc Bonferroni tests. Unpaired $t$-tests were used to assess the influence of estrous stage on PE values for the colon, uterus, or bladder due to its treatment with MO. Pearson correlation coefficient calculations were done to compare organ weights and PE values. Alpha levels were set at 0.05. Data are presented as means ± SE.

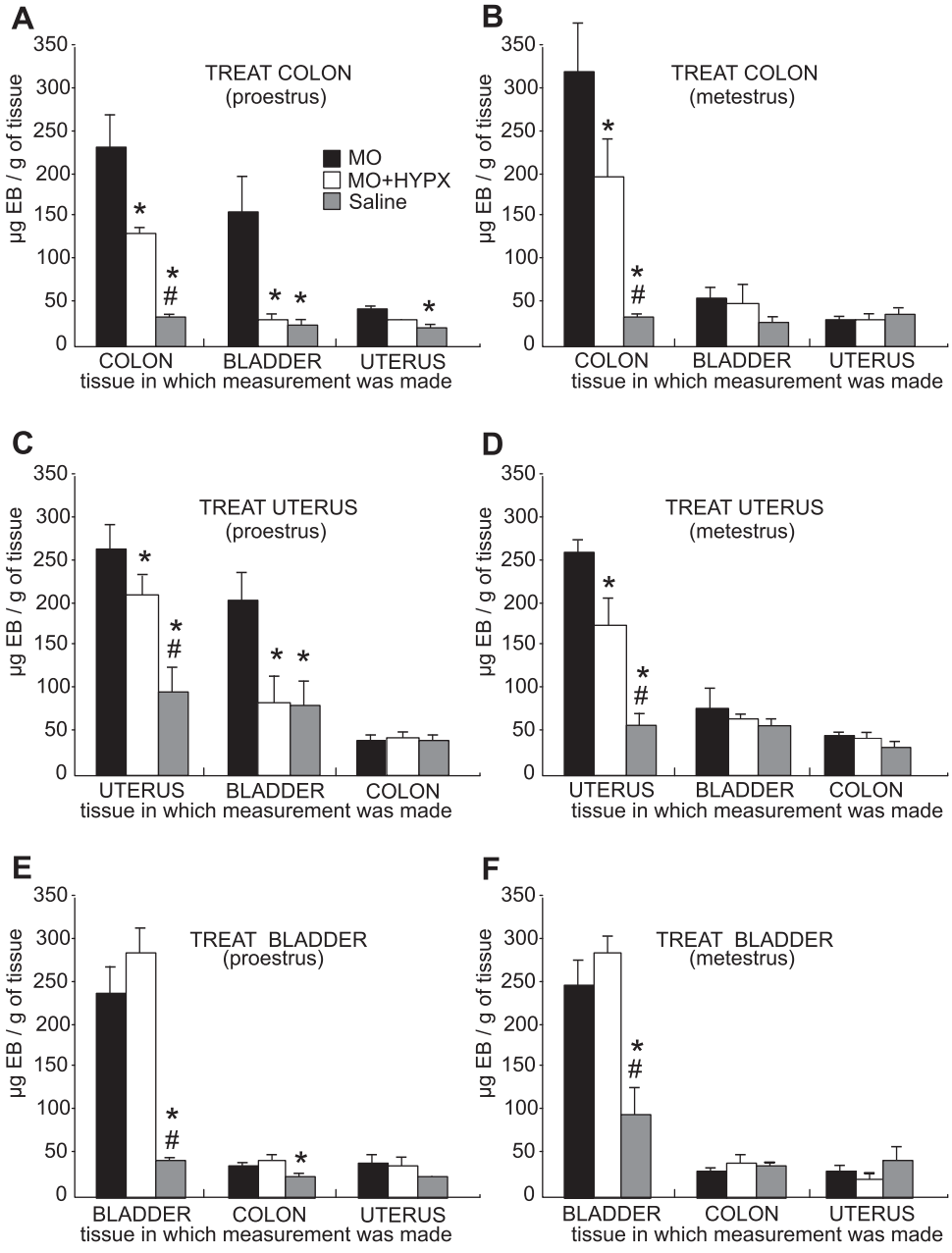


Fig. 1. Extravasation of Evans Blue dye in the colon, uterine horn, and bladder. The graphs are arrayed to enable visualization of protein extravasation (PE) values in the colon, bladder, and uterine horn after treating one of the organs with mustard oil (MO), MO following hypogastric neurectomy (MO+HYPX), or saline. Results from rats in proestrus are shown in the left column of graphs (*A*, *C*, *E*). Results from rats in metestrus are shown in the right column of graphs (*B*, *D*, *F*). A and B: illustration of PE values for all three organs from rats whose colon was treated with either MO, MO+HYPX, or saline. *C* and *D*: illustration of PE values for all three organs from rats whose uterine horn was treated with either MO, MO+HYPX, or saline. *E* and *F*: illustration of PE values for all three organs from rats whose bladder was treated with either MO, MO+HYPX, or saline. Note that for each pair of graphs, PE values for the treated organ are placed as the left triplet of bars. The middle and right triplet of bars show PE values for the untreated organs from those rats. For each organ: *significantly different from MO-treated group; #significantly different from MO+HYPX-treated group.

## RESULTS

*Effects of treating the colon.* The effects of different treatments of the colon on PE values differed significantly in both proestrus [$F(2,18) = 22.8$, $P < 0.001$] and metestrus [$F(2,18) = 10.45$, $P = 0.001$]. Furthermore, the within-subject effects of treating the colon differed significantly across organs in both proestrus [$F(1,18) = 16.42$, $P = 0.001$] and metestrus [$F(1,18) = 34.0$, $P < 0.001$]. PE values for the MO-treated colon in proestrus and the MO-treated colon in metestrus did not differ significantly.

As shown in Fig. 1, *A* and *B*, further analysis showed that PE values for the MO-treated colon were significantly greater than PE values for the saline-treated colon in both estrous stages. In proestrus (Fig. 1*A*), but not in metestrus (Fig. 1*B*), PE values for the untreated bladder from rats whose colon had been treated with MO were significantly greater than PE values for the untreated bladder from rats whose colon had been treated with saline. A similar but much less significant cross-organ effect occurred in the untreated uterine horn.

In other words, for rats in proestrus, treating the colon with MO increased extravasation not only in the MO-treated colon but also in the untreated bladder and to a much lesser extent in the untreated uterine horn. Although the amount of extravasation in the inflamed colon was the same in metestrus as it was in proestrus, the cross-organ effects on the bladder and uterine horn did not occur in rats in metestrus.

As also shown in Fig. 1, *A* and *B*, HYPX significantly reduced PE values in the MO-treated colon in proestrus and metestrus, but the values remained significantly greater than those in the saline-treated colon. In other words, in both

proestrus and metestrus, HYPX reduced but did not eliminate the direct effects of MO on the colon.

HYPX did, however, eliminate the cross-organ effects of MO treatment of the colon on the untreated proestrous bladder. In other words, in rats in proestrus whose colon had been treated with MO, HYPX significantly reduced PE values in the untreated bladder to levels insignificantly different from PE values in the untreated bladder of proestrous rats whose colon had been treated with saline.

Some of these effects are shown in Fig. 2, which provides photographs taken from four different rats: three rats in proestrus whose colon had been treated with saline, MO, or MO+HYPX and one rat in metestrus whose colon had been treated with MO. Please note that the size of the bladder differs in the four pictures because the bladders are viewed from different angles and some bladders contain more urine than others. What is notable is that the bladder is bluer in the three situations in which the colon was inflamed with MO than in the situation in which the colon was treated with saline.

*Effects of treating the uterine horn.* As in the colon-treated groups, the effects of different treatments of the uterine horn on PE values differed significantly in both proestrus [$F(2,15) = 9.39$, $P = 0.002$] and metestrus [$F(2,15) = 8.82$, $P = 0.003$]. Furthermore, the within-subject effects of treating the uterine horn differed significantly across organs in both proestrus [$F(1,15) = 10.67$, $P = 0.005$] and metestrus [$F(1,15) = 43.27$, $P < 0.001$]. PE values for the MO-treated uterine horn in proestrus and metestrus did not differ significantly.

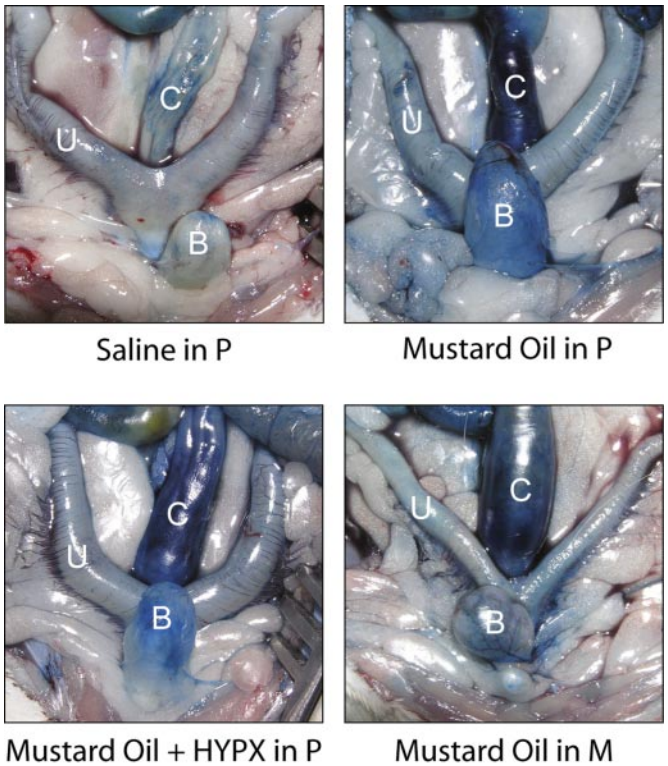


Fig. 2. Photographs of examples showing how different treatments of the colon affect the extravasation of Evans Blue dye in the bladder when the rat was in proestrus (P) or metestrus (M). Photographs were taken ~2 h after injection of Evans Blue dye. Note the following: *1*) that the bladder appears the most "blue," i.e., shows the most extravasation in the rat treated with MO in P (upper right photo) when compared with bladders in the other examples; and *2*) that the inflamed area of the colon appears equally dark in the 3 rats whose colon was treated with MO, regardless of estrous stage. B, bladder; C, colon; U, uterine horn.

As shown in Fig. 1, *C* and *D*, further analysis showed that PE values for the MO-treated uterine horn were significantly greater than PE values for the saline-treated uterine horn in both estrous stages. In proestrus (Fig. 1*C*), but not in metestrus (Fig. 1*D*), PE values for the untreated bladder from rats whose uterine horn was treated with MO were significantly greater than PE values for the untreated bladder from rats whose uterine horn had been treated with saline.

Although there was no evidence in either stage for a cross-organ effect of treating the uterine horn with MO on the untreated colon, it is possible that if the preparation of the uterine horn had not inflamed it slightly (see *Assessment of the interpretative consequences of potential differences between the methods used to prepare each organ for treatment*), PE values in the colon from rats in which the uterine horn had been treated with saline would have been smaller. If so, a small significant cross-organ effect of treating the uterine horn with MO on PE in the colon might have been observed (i.e., similar to the small effect described above of treating the colon with MO on PE in the uterine horn).

In other words, for rats in proestrus, treating the uterine horn with MO increased extravasation not only in the MO-treated uterine horn, but also in the untreated bladder (and might have done so to a much smaller extent in the untreated colon). Although the amount of extravasation in the uterine horn was the same in proestrus and metestrus, the cross-organ effect on the bladder did not occur in rats in metestrus.

As also shown in Fig. 1, *C* and *D*, HYPX significantly reduced the PE values for the MO-treated uterine horn in both proestrus and metestrus, but the values remained significantly greater than those in the saline-treated uterine horn. In other words, in both proestrus and metestrus, HYPX reduced but did not eliminate the effects of MO on the uterine horn.

HYPX did, however, eliminate the cross-organ effects of MO treatment of the uterine horn on the untreated bladder. In other words, in rats in proestrus whose uterine horn had been treated with MO, HYPX significantly reduced PE values in the untreated bladder to levels insignificantly different from levels in the untreated bladder of proestrous rats whose uterine horn had been treated with saline.

*Effects of treating the bladder.* As in the colon- and uterine horn-treated groups, the effects of different treatments of the bladder on PE values differed significantly in both proestrus [$F(2,16) = 21.8$, $P < 0.001$] and metestrus [$F(2,15) = 11.4$, $P = 0.001$]. Furthermore, the within-subject effects of treating the bladder differed significantly across organs in both proestrus [$F(1,16) = 115.0$, $P < 0.001$] and metestrus [$F(1,15) = 139.2$, $P < 0.000$]. PE values for the MO-treated bladder in proestrus and metestrus did not differ significantly.

As shown in Fig. 1, *E* and *F*, further analysis showed that PE values for the MO-treated bladder were significantly greater than PE values for the saline-treated bladder in both estrous stages. In proestrus (Fig. 1*E*), but not metestrus (Fig. 1*F*), PE values for the untreated colon from rats whose bladder had been treated with MO were slightly, but significantly, greater than PE values for the untreated colon from rats whose bladder had been treated with saline. There was no evidence in either

stage for a cross-organ effect of treating the bladder with MO on the untreated uterine horn.

In other words, for rats in proestrus, treating the bladder with MO increased PE values not only in the MO-treated bladder, but also to a very small extent in the untreated colon (but not in the untreated uterine horn). This small bladder-to-colon effect did not occur in rats in metestrus.

As also shown in Fig. 1, *E* and *F*, unlike the situation after treatments of the colon or uterine horn, HYPX had no influence on PE values for the MO-treated bladder in either proestrus or metestrus. Furthermore, HYPX did not eliminate the small influence that MO treatment of the bladder had on the untreated colon in proestrus.

*Assessment of the interpretative consequences of potential differences between the methods used to prepare each organ for treatment.* Treatment of each organ with saline was used to control for the potential influence of the manner in which each organ was prepared for its treatment on its vascular permeability. Thus the difference between groups for a specific treated organ can only be due to the inflammation with MO (with or without HYPX), regardless of how that organ was prepared for treatment.

On the other hand, although every attempt was made to prepare the different organs in as similar a manner as possible, preparation of the uterine horn differed from that of the colon and bladder because the uterine horn was ligated at its junction with the body of the uterus (to prevent leakage), whereas the other two organs were not ligated directly. This situation created the possibility that PE values for saline treatment of the uterus might be elevated compared with PE values for the other saline-treated organs. If so, conclusions concerning possible differences between the three organs in their cross-organ effects could be affected.

To determine whether such a situation was in fact the case, we used internal controls to analyze the data shown in Fig. 1 for each organ treated. The logic was as follows. If we consider one organ, say *organ X*, it is possible to compare PE values of untreated *organ X* taken from subjects in which *organ Y* or *Z* was treated with saline with PE values of the saline-treated *organ X*. For example, the PE value for the untreated colon when the uterus or bladder was treated with saline was 34.95 ± 4.25 SE. The PE value for the saline-treated colon was nearly identical at 34.01 ± 3.8 SE. These values did not differ significantly (unpaired *t*-test; $P = 0.80$). The same lack of difference occurred in the bladder (unpaired *t*-test; $P = 0.21$). Therefore, preparation of the colon or bladder did not inflame those two organs.

The situation was different, however, for the saline-treated uterine horn. The PE values for the saline-treated uterine horn were 77.91 ± 16.3 SE, whereas PE values for the untreated uterine horn harvested from rats in which the colon and bladder had been treated with saline were 32.56 ± 3.7 SE. An unpaired *t*-test indicates that this difference is statistically significant ($P = 0.02$). To confirm this difference and to determine whether our internal control logic used above was valid, we compared the PE values for the untreated uterine horn with PE values for eight uterine horn samples taken from rats in another study in which no other pelvic organ had been inflamed. The PE values for that other group were 39.59 ± 8.29 SE, which did not differ significantly from the PE values of samples taken from untreated uterine horn samples in the present study ($P = 0.47$). Thus it appears that preparation of the uterine horn did indeed produce a small amount of inflammation (compared with two- to threefold greater inflammation produced in the MO-treated uterus) and that our logic was valid.

Overall, this analysis indicates that the small amount of inflammation in the saline-treated uterine horn might have had minor consequences for conclusions concerning differences in the cross-organ effects between the three different organs. As described above, it might have allowed us to conclude that uterine inflammation produced a small cross-organ effect on PE in the colon (similar to the small effect that inflammation of the colon had on PE in the uterus).

*Tissue weights.* Weights of each MO-treated organ were consistently significantly heavier than the weights of the corresponding saline-treated organ or untreated organ. Thus for the bladder, mean weights for the MO-treated bladder (regardless of whether HYPX had been done) vs. the saline or untreated bladders were 146 ± 10.4 g and 102 ± 3.4 g, respectively ($P < 0.001$, *t*-test). For the colon, mean weights for the MO-treated colon vs. the saline or untreated colon were 197 ± 27.1 g and 104.4 ± 3.4 g, respectively ($P < 0.001$, *t*-test). For the uterine horn, mean weights for the MO-treated uterine horn vs. the saline or untreated uterine horn were 83.7 ± 27.1 and 71.27 ± 3.4 g, respectively ($P < 0.05$, *t*-test).

On the other hand, weights for untreated organs did not differ significantly across groups. Thus, taking the bladder as an example, one-way ANOVA values when comparing weights of the untreated bladder from groups in which the colon had been treated with MO, MO+HYPX, or saline were not significant in either proestrus [$F(2,18) = 1.048$, $P = 0.375$] or metestrus [$F(2,18) = 0.89$, $P = 0.428$]. Similarly, one-way ANOVA values when comparing weights of the untreated bladder from groups in which the uterus had been treated with MO, MO+HYPX, or saline were not significant in either proestrus [$F(2,15) = 1.47$, $P = 0.259$] or metestrus [$F(2,15) = 1.59$, $P = 0.236$]. There was a similar lack of significance between groups for the untreated colon and untreated uterus.

*Correlations between weight and PE values of untreated organs.* Despite the fact that the weights of all three of the untreated organs did not vary across groups, there were differences between the three untreated organs with respect to the correlation between their weights and PE values. Thus for the untreated bladder in proestrus, the correlations between bladder tissue weights and PE values were significant when the either the colon or uterus was treated with MO, MO+HYPX, or saline (for treatment of the colon, $r = 0.412$, $P < 0.05$; for treatment of the uterus, $r = 0.74$, $P < 0.001$). These correlations were not significant for the untreated bladder in metestrus. For the untreated colon and uterus, none of the correlations was significant. In other words, for the untreated bladder, bladder weight and PE values correlated significantly when the colon or uterus was treated in proestrus, but not when the colon or uterus was treated in metestrus. In contrast, there were no significant correlations between weight and PE for the untreated colon or untreated uterus. Overall, therefore, the pattern of significant correlations between weights and PE paralleled the pattern of significant cross-organ effects assessed by PE.

## DISCUSSION

This study produced four new findings. First, inflammation of the uterine horn, like inflammation of the colon, gave rise to robust signs of inflammation in the bladder. Second, the cross-organ uterine horn-to-bladder and colon-to-bladder effects were influenced by the estrous cycle, i.e., they occurred in proestrus but not metestrus. Third, the hypogastric nerve appears to be involved in these cross-organ effects because HYPX eliminated them. Fourth, the bladder appears more vulnerable than the colon and uterine horn are to some types of cross-organ influences.

More specifically, we found that acute inflammation of the colon or uterine horn in proestrus (estradiol levels high; progesterone levels low) but not metestrus (estradiol and progesterone levels low) increased extravasation in the untreated bladder. Again in proestrus, but not metestrus, bladder inflammation produced a much smaller but significant increase of extravasation in the uninflamed colon, and colon inflammation produced a much smaller but significant increase of extravasation in the uninflamed uterus. The cross-organ effects were selective, however, because inflammation of the uterine horn had no influence on extravasation in the untreated colon (but see further below), and inflammation of the bladder had no influence on extravasation in the untreated uterine horn.

*Cross-organ effects.* Our findings of cross-organ effects between pelvic organs in the gastrointestinal, urinary, and reproductive tracts are consistent with earlier reports. Previously, we showed that acute bladder inflammation in proestrus decreases contractions of the untreated uterine horn (22). Acute bladder inflammation also reduces the efficacy of cannabinoid agonists on the uterine horn (21). In addition, others have recently reported that acute cystitis increases abdominal wall muscle activity that is evoked by distention of the untreated colon (55) and, conversely, that acute, as well as chronic, colon irritation induces bladder overactivity (41, 55).

The results on cross-organ effects in the pelvis are also consistent with reports of cross-organ effects between pelvic organs and organs located in more widespread areas of the body. For example, surgically induced endometriosis in the upper abdomen, a chronic condition, reduces bladder micturition thresholds measured >1 mo postoperatively (50). Similarly, in awake rats, surgically induced abdominal endometriosis at a similar postoperative period exacerbates pain behaviors associated with a ureteral calculosis (26) and increases escape responses to vaginal distention (18).

Of relevance to this discussion are some differences between the results here and those of a recent report by Malykhina et al. (43). This group, like Pezzone et al. (54), has evidence consistent with (but not proof for) the possibility that a small proportion of single afferent fibers may branch to supply the colon and bladder, which in turn is consistent with the observations here of cross-organ effects between the colon and bladder (see further discussion below). However, the results here are inconsistent with another part of the Malykhina groups' study (43). In this other part of their study, the colon was inflamed with trinitrobenzene sulfonic acid (TNBS), and either 3 or 30 days later, signs of inflammation of the both the inflamed colon and the uninflamed bladder were assessed using a MPO activity assay. Although MPO activity had increased in the colon by 3 days (and was recovered by 30 days), it had *not* increased in the bladder at either 3 or 30 days after colon inflammation.

Part of the reason for the difference in findings could be the different assays (MPO vs. dye extravasation) or inflammatory agents (MO or TNBS) used by them or us. An alternative, and more likely explanation, however, involves the timing of the assays. Here, extravasation was measured 2 h after inflammation (i.e., after an acute inflammation), whereas the Malykhina groups' measures (43) were made 3 days after inflammation (i.e., after a chronic inflammation). It is possible, therefore, that cross-organ induction of an "indirect" sign of inflammation (MPO or PE) might be limited to the acute effects of the inflammation. If this explanation is correct, it has implications for the process by which cross-organ effects that are initiated by pathology in one of the organs are maintained over time. Further studies on the development of cross-organ effects are therefore warranted (for example, see Ref. 71).

*Vulnerability of bladder to cross-system effects.* Among the three pelvic organs studied here, the bladder appeared the most vulnerable to cross-system effects and the least effective in inducing them. Thus a substantial amount of bladder extravasation was induced by inflammation of either the colon or uterine horn in proestrus, and this extravasation was accompanied by increases in bladder weight. In contrast, either no cross-organ effects or only small and perhaps biologically insignificant cross-organ effects were induced in the colon or uterine horn.

Given the robust effects of bladder inflammation on colon and uterine horn activity reported, respectively, by Pezzone et al. (55) and Dmitrieva et al. (22), the small effects observed here on the colon and uterine horn were surprising. There are several possible explanations. The most obvious is a difference in the outcome measures; i.e., bladder and uterine contractility by the earlier authors vs. vascular permeability here. The PE measure used here might not have been sensitive enough to detect possibly more subtle changes in the colon and uterine horn. Another possibility is a difference in timing. We studied the bladder-to-colon effects 2 h after inflammation, whereas Pezzone et al. (55) studied them ~60 min after irritation, and Dmitrieva et al. (22) found that the bladder-to-uterus effects were maximal at times later than our measurements here (4 h). A third possibility is a difference in the agents used for irritation/inflammation: protamine sulfate followed by potassium chloride or 50% turpentine, respectively, vs. 10% MO here.

It is, of course, also possible that the bladder is indeed more vulnerable to cross-organ influences and less effective than are the colon and uterine horn in inducing certain types of cross-organ effects, such as increased PE. This possibility is supported by the fact that, whereas HYPX reduced extravasation in the inflamed colon and uterine horn, it failed to reduce extravasation in the inflamed bladder. Furthermore, unlike the situation after colon or uterine horn inflammation, HYPX did not eliminate the bladder's small effect on the colon. These findings suggest that the small colon-to-bladder and uterine horn-to-bladder effects observed here depend on both sensory and autonomic efferent fibers in the hypogastric nerve and that this mechanism is not engaged in the opposite direction when the bladder is inflamed. Further support for the particular vulnerability of the bladder to indirectly induced dysfunction comes from the work of Jasmin and Janni (35), who showed

that viral infection of the spinal cord induced by innoculation of pseudorabies virus into tail muscles of the rat produces a neurogenic inflammation limited to the bladder.

*Mechanisms of cross-organ effects.* The fact that HYPX eliminated the effects of colon and uterine horn inflammation on the bladder suggests that the hypogastric nerve contributes to the cross-organ effects. Thus the results support earlier findings implicating involvement of the hypogastric nerve in the consequences of inflammation (13, 22, 69) and further suggest that one of these consequences includes cross-organ effects.

Given that all three organs are innervated by sensory and autonomic fibers in the hypogastric nerve (40), there are at least five potential and, importantly, compatible mechanisms. These mechanisms are diagrammed in Fig. 3.

Two mechanisms involve only afferent fibers (Fig. 3, *A* and *B*). In the first, the cross-organ effects are brought about by neurogenic axon reflexes (61, 67) occurring in single hypogastric sensory axons that branch to supply more than one pelvic organ (Fig. 3*A*). To explain the results here, this mechanism would require evidence for the existence of single sensory fibers that branch to supply the colon and bladder, the uterine horn and bladder, the colon and uterine horn, or all three organs. Although evidence consistent with the existence of such branching for the bladder and colon has recently been found using double-labeling methods (43, 54) and Ustinova et al. (70) recently reported that afferent fibers that supply the bladder are sensitized to noxious stimuli after colonic inflammation, there is no evidence concerning the potential existence of axons that branch to supply the uterine horn and bladder or the uterine horn and colon.

The second afferent-only mechanism (Fig. 3*B*) is the dorsal root reflex. Here, presynaptic axoaxonal effects of hypogastric afferents from the inflamed organ could, via an inhibitory interneuron in the spinal cord, sensitize and antidromically activate other hypogastric afferents from an uninflamed organ, thereby producing extravasation in the uninflamed organ (76). Either this mechanism or branching sensory fibers could also explain recent findings by Ustinova et al. (70) in which the sensitization of bladder afferent fibers by colon stimulation was blocked by bladder denervation. In support of this possibility are findings by Qin et al. (58) in which it was shown that one population of spinal neurons responding only to bladder input was sensitized when the colon was chronically inflamed. In this case, however, branching (Fig. 3*A*) would be an unlikely mechanism, because, if so, then the neurons should have responded to both colon and bladder stimulation. Further support for the dorsal root reflex mechanism comes from studies done by Jasmin and colleagues (35, 36) who found that a viral infection of central nervous system neurons neighboring those that process bladder information induces bladder inflammation.

The three additional mechanisms diagrammed in Fig. 3 involve both afferent fibers and postganglionic fibers from the pelvic ganglion (Fig. 3, *C–E*) and rely on three published facts. First, there is extensive convergence of pelvic organ input on neurons in the spinal cord and brain (7, 24, 38). Second, preganglionic fibers in the hypogastric nerve synapse not only on neurons in the inferior mesenteric ganglion but also on neurons in the pelvic ganglion (23, 68), i.e., the pelvic ganglion receives preganglionic input from fibers traveling in both the hypogastric nerve derived from T13/L1 and the pelvic nerve derived from L6-S2. Third, whereas centrally generated activation of postganglionic sympathetic fibers fails to elicit PE (48), activation of postganglionic parasympathetic efferents does elicit extravasation (3, 20). Thus it is possible that input from the inflamed organ via the hypogastric nerve activates neurons in the dorsal horn of T13/L1 that in turn eventually activate postganglionic neurons in the pelvic ganglion via three different multisynaptic routes: *1*) preganglionic neurons in T13/L1 (Fig. 3C); *2*) intraspinal connections (73) to preganglionic neurons in L6-S2 (Fig. 3*D*); and *3*) ascending input to brain stem (e.g., 32, 59, 60) that in turn send descending fibers to preganglionic neurons in L6-S2 (Fig. 3*E*) or T13/L1 (not shown).

All of the mechanisms shown in Fig. 3 could contribute to pelvic organ interactions here, as well as those observed by others, such as the reciprocal colon-bladder cross-sensitization assessed by organ reflexes (55) and changes in excitability of bladder-colon convergent dorsal root ganglion neurons induced by colon inflammation (43). However, with respect to the present findings in which extravasation was the outcome measure, there are four reasons why the first, purely peripheral mechanism of branching sensory axons might not be the dominant one (Fig. 3*A*). First, because HYPX was performed distal to the IMG, the branches would have to originate proximal to the IMG (as shown in Fig. 3*A*). Anatomical studies, however, suggest that the branches occur distally (33). Second, the effects observed here were not reciprocal; i.e., if the cross-system effects were due to axon reflexes in branches of the same sensory afferents supplying the two organs, then the effects should go both ways. Third, it has never been reported that a peripheral sensory fiber can respond to stimulation of more than one pelvic organ, even when attempts have been made to discover such responses (e.g., 10, 12, 13, 25). Fourth, it is possible to elicit bladder inflammation by a purely central dysfunction (35).

*Influence of estrous stage.* The estrous influences on the cross-system effects observed here suggest that the effects are influenced by ovarian hormones, possibly estradiol. Although most previous studies of cross-organ influences between the colon and bladder have not investigated such influences (e.g., 41, 43, 54, 55, 57), the present results are consistent with findings showing that the severity of vaginal hyperalgesia induced by abdominal endometriosis correlates with circulating levels of estradiol (18). In that study, the hyperalgesia was greatest in proestrus (high level of estradiol and low level of progesterone) and was completely alleviated in estrus (very low estradiol and progesterone), a result similar to that observed here. Furthermore, Jasmin and Janni (35) have reported that extravasation in the bladder of rats with centrally induced neurogenic cystitis is greater in females than males and prevented in both sexes by gonadectomy.

Because estrogen receptors are present on primary sensory afferents of pelvic organs (52), estradiol could exert an effect on PE by influencing antidromic activation of sensory terminals. Another possibility is that estradiol influences PE directly by modulating release of inflammatory mediators from peripheral mast cells. Thus it has been shown that human gastrointestinal and bladder mast cells express estrogen receptors (37, 42, 51) and that estradiol enhances degranulation and hista-

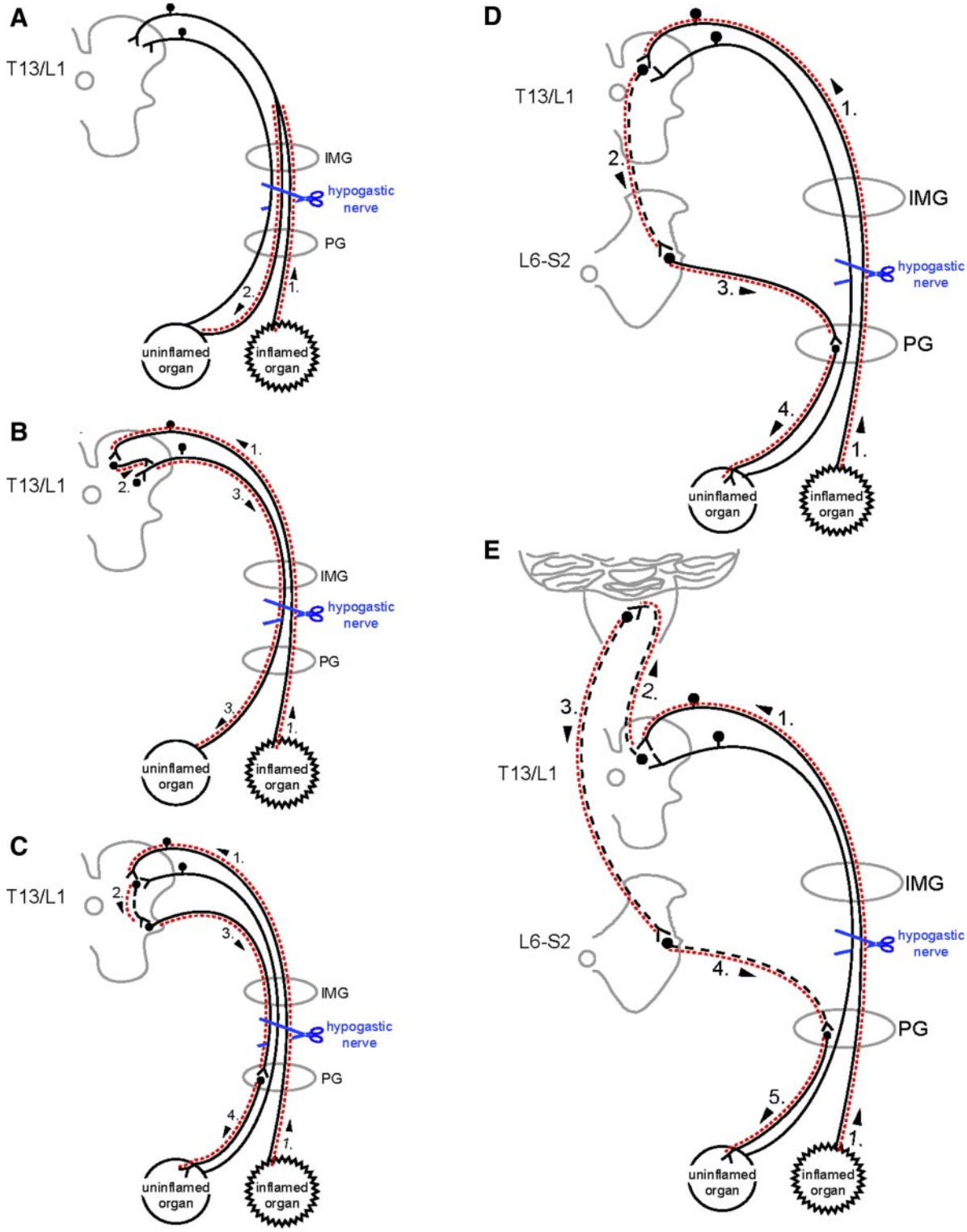


Fig. 3. Five compatible mechanisms by which hypogastric nerve fibers could contribute to the process by which inflammation of one organ induces signs of inflammation (protein extravasation) in another, uninflamed organ. *A*: branching sensory afferents. *B*: dorsal root reflex (adapted from Ref. 76). *C*: multisynaptic route involving sensory afferents from the inflamed organ to the T13/L1 segment of the cord, followed by output from preganglionic fibers in the T13/L1 segment to postganglionic fibers in the pelvic ganglion that innervate the uninflamed organ. *D*: multisynaptic route involving sensory afferents from the inflamed organ to the T13/L1 segment of the cord, followed by output from preganglionic fibers in the L6-S2 segments to postganglionic fibers in the pelvic ganglion that innervate the uninflamed organ. In this case, the multisynaptic route includes intraspinal connections between the T13/L1 and L6-S2 segments of the spinal cord. *E*: multisynaptic route involving sensory afferents from the inflamed organ to the T13/L1 segment of the cord, followed by output from preganglionic fibers in the L6-S2 segments to postganglionic fibers in the pelvic ganglion that innervate the uninflamed organ. In this case, the multisynaptic route includes ascending connections from spinal cord to brain and descending connections from brain to L6-S2 segments. See *Mechanisms of cross-organ effects* for more detail. Dotted red lines and black arrows indicate the direction and sequence of information flow. Dashed black lines indicate multisynaptic connections. IMG, inferior mesenteric ganglion; PG, pelvic ganglion.

mine secretion from mast cells (29, 42, 72) and increases sensitivity of mast cells to substance P stimulation (14).

While compelling, the failure to find estrous differences in either the amount of extravasation or the effect of HYPX on extravasation in the inflamed organ makes these peripheral explanations less likely. Another possibility, therefore, is that the estrous influences on cross-organ effects are exerted centrally. Potential central location(s) of such effects are widespread. Estrogen receptors have been found on neurons located throughout the spinal cord and brain that influence the uterus and other organs (53, 64).

*Clinical relevance.* The cross-organ affects between pelvic organs observed here that were induced by acute inflammation, when considered together with findings by others discussed above of cross-organ effects induced by either acute or chronic pathology in either nearby or remote organs, are likely relevant to the surprisingly frequent clinical situation in which several painful disorders cooccur. Examples include the cooccurrence of dysmenorrhea, irritable bowel disorder, interstitial cystitis, and vulvodynia (1, 65), as well as the distressing disorder, multiple organ dysfunction syndrome (44, 45). The estrous influence on the cross-system effects observed here is also consistent with the fact that symptom severity in these disorders is influenced by sex steroid hormones (e.g., 2, 46, 56).

For understanding this relevance, however, it is clear that further studies are needed, particularly those using functional outcome measures (e.g., organ motility in awake animals) and other measures of inflammation (e.g., MPO, see Ref. 43) to assess the relevance and significance of the cross-organ effects for organ function. Further studies are also needed to improve understanding of how the cross-organ effects occur (e.g., peripheral or central or both; whether other nerves such as pelvic and vagus nerves are involved), as well as how the effects develop over time and how they are modulated by reproductive status.

## ACKNOWLEDGEMENT

We thank John Chalcroft for help with the illustrations.

## GRANTS

This study was supported by National Institutes of Health Grants R21-DK-063967 and R01-NS-11892 and the Fishbein Foundation of the Interstitial Cystitis Association.

## REFERENCES

1. **Alagiri M, Chottiner S, Ratner V, Slade D, and Hanno PM.** Interstitial cystitis: unexplained associations with other chronic diseases and pain syndromes. *Urology* 49, *Suppl* 5a: 52–57, 1997.
2. **Ananthakrishnan P, Cohen DB, Xu da Z, Lu Q, Feketeova E, and Deitch EA.** Sex hormones modulate distant organ injury in both a trauma/hemorrhagic shock model and a burn model. *Surgery* 137: 56–65, 2005.
3. **Asztely A, Havel G, and Ekstrom J.** Vascular protein leakage in the rat parotid gland elicited by reflex stimulation, parasympathetic nerve stimulation and administration of neuropeptides. *Regul Pept* 77: 113–120, 1998.
4. **Baez MA, Brink TS, and Mason P.** Roles for pain modulatory cells during micturition and continence. *J Neurosci* 25: 384–394, 2005.
5. **Becker JB, Arnold AP, Berkley KJ, Blaustein JD, Eckel LA, Hampson E, Herman JP, Marts S, Sadee W, Steiner M, Taylor J, and Young E.** Strategies and methods for research on sex differences in brain and behavior. *Endocrinology* 146: 1650–1673, 2005.
6. **Bereiter DA, Okamoto K, and Bereiter DF.** Effect of persistent monoarthritis of the temporomandibular joint region on acute mustard oil-induced excitation of trigeminal subnucleus caudalis neurons in male and female rats. *Pain* 117: 58–67, 2005.
7. **Berkley KJ.** A life of pelvic pain. *Physiol Behav* 86: 272–280, 2005.
8. **Berkley KJ and Hubscher CH.** Are there separate central nervous system pathways for touch and pain? *Nat Med* 1: 766–773, 1995.
9. **Berkley KJ, Guilbaud G, Benoist JM, and Gautron M.** Responses of neurons in and near the thalamic ventrobasal complex of the rat to stimulation of uterus, cervix, vagina, colon, and skin. *J Neurophysiol* 69: 557–568, 1993.
10. **Berkley KJ, Hotta H, Robbins A, and Sato Y.** Functional properties of afferent fibers supplying reproductive and other pelvic organs in pelvic nerve of female rat. *J Neurophysiol* 63: 256–272, 1990.
11. **Berkley KJ, Hubscher CH, and Wall PD.** Neuronal responses to stimulation of the cervix, uterus, colon, and skin in the rat spinal cord. *J Neurophysiol* 69: 545–556, 1993.
12. **Berkley KJ, Robbins A, and Sato Y.** Afferent fibers supplying the uterus in the rat. *J Neurophysiol* 59: 142–163, 1988.
13. **Berkley KJ, Robbins A, and Sato Y.** Functional differences between afferent fibers in the hypogastric and pelvic nerves innervating female reproductive organs in the rat. *J Neurophysiol* 69: 533–544, 1993.
14. **Bradesi S, Eutamene H, Theodorou V, Fioramonti J, and Bueno L.** Effect of ovarian hormones on intestinal mast cell reactivity to substance P. *Life Sci* 68: 1047–1056, 2001.
15. **Bradshaw HB and Berkley KJ.** Estrous changes in responses of rat gracile nucleus neurons to stimulation of skin and pelvic viscera. *J Neurosci* 20: 7722–7727, 2000.
16. **Bradshaw HB, Berkley KJ.** The influence of ovariectomy with or without estrogen replacement on responses of rat gracile nucleus neurons to stimulation of hindquarter skin and pelvic viscera. *Brain Res* 986: 82–90, 2003.
17. **Bruggemann J, Shi T, and Apkarian AV.** Squirrel monkey lateral thalamus. II. Viscerosomatic convergent representation of urinary bladder, colon, and esophagus. *J Neurosci* 14: 6796–6814, 1994.
18. **Cason AM, Samuelsen CL, and Berkley KJ.** Estrous changes in vaginal nociception in a rat model of endometriosis. *Horm Behav* 44: 123–131, 2003.
19. **Collins JJ, Lin CE, Berthoud HR, and Papka RE.** Vagal afferents from the uterus and cervix provide direct connections to the brainstem. *Cell Tissue Res* 295: 43–54, 1999.
20. **Delepine L and Aubineau P.** Plasma protein extravasation induced in the rat dura mater by stimulation of the parasympathetic sphenopalatine ganglion. *Exp Neurol* 147: 389–400, 1997.
21. **Dmitrieva N and Berkley KJ.** Contrasting effects of WIN 55212–2 on motility of the rat bladder and uterus. *J Neurosci* 22: 7147–7153, 2002.
22. **Dmitrieva N, Johnson OL, and Berkley KJ.** Bladder inflammation and hypogastric neurectomy influence uterine motility in the rat. *Neurosci Lett* 313: 49–52, 2001.
23. **Felix B, Catalin D, Miolan JP, and Niel JP.** Integrative properties of the major pelvic ganglion in the rat. *J Auton Nerv Syst* 69: 6–11, 1998.
24. **Foreman RD.** Integration of viscerosomatic sensory input at the spinal level. *Prog Brain Res* 122: 209–221, 2000.
25. **Gebhart GF.** Peripheral contributions to visceral hyperalgesia. *Can J Gastroenterol* 13, *Suppl A*: 37A–41A, 1999.
26. **Giamberardino MA, Berkley KJ, Affaitati G, Lerza R, Centurione L, Lapenna D, and Vecchiet L.** Influence of endometriosis on pain behaviors and muscle hyperalgesia induced by a ureteral calculosis in female rats. *Pain* 95: 247–257, 2002.
27. **Godec CJ, Cass AS, and Ruiz E.** Another aspect of acute urinary retention in young patients. *Ann Emerg Med* 11: 471–474, 1982.
28. **Gold MS and Traub RJ.** Cutaneous and colonic rat DRG neurons differ with respect to both baseline and PGE2-induced changes in passive and active electrophysiological properties. *J Neurophysiol* 91: 2524–2531, 2004.
29. **Harnish DC, Albert LM, Leathurby Y, Eckert AM, Ciarletta A, Kasaian M, and Keith JC Jr.** Beneficial effects of estrogen treatment in the HLA-B27 transgenic rat model of inflammatory bowel disease. *Am J Physiol Gastrointest Liver Physiol* 286: G118–G125, 2004.
30. **Hart BL.** Normal behavior and behavioral problems associated with sexual function, urination, and defecation. *Vet Clin North Am* 4: 589–606, 1974.
31. **Honda CN.** Visceral and somatic afferent convergence onto neurons near the central canal in the sacral spinal cord of the cat. *J Neurophysiol* 53: 1059–1078, 1985.

32. **Hubscher CH, Kaddumi EG, and Johnson RD.** Brain stem convergence of pelvic viscerosomatic inputs via spinal and vagal afferents. *Neuroreport* 15: 1299–1302, 2004.
33. **Hulsebosch CE and Coggeshall RE.** An analysis of the axon populations in the nerves to the pelvic viscera in the rat. *J Comp Neurol* 211: 1–10, 1982.
34. **Jänig W and McLachlan EM.** Organization of lumbar spinal outflow to distal colon and pelvic organs. *Physiol Rev* 67: 1332–1404, 1987.
35. **Jasmin L and Janni G.** Experimental neurogenic cystitis. *Adv Exp Med Biol* 539: 319–335, 2003.
36. **Jasmin L, Janni G, Manz HJ, and Rabkin SD.** Activation of CNS circuits producing a neurogenic cystitis: evidence for centrally induced peripheral inflammation. *J Neurosci* 18: 10016–10029, 1998.
37. **Jiang YA, Zhang YY, Luo HS, and Xing SF.** Mast cell density and the context of clinicopathological parameters and expression of p185, estrogen receptor, and proliferating cell nuclear antigen in gastric carcinoma. *World J Gastroenterol* 8: 1005–1008, 2002.
38. **Kaddumi EG and Hubscher CH.** Convergence of multiple pelvic organ inputs in the rat rostral medulla. *J Physiol* 572: 393–405, 2006.
39. **Kandel ER, Schwartz JH, and Jessell TM.** *Principles of Neural Science* (4th ed.). New York: McGraw-Hill, 2000.
40. **Keast JR and de Groat WC.** Segmental distribution and peptide content of primary afferent neurons innervating the urogenital organs and colon of male rats. *J Comp Neurol* 319: 615–623, 1992.
41. **Lamb K, Zhong F, Gebhart GF, and Bielefeldt K.** Experimental colitis in mice and sensitization of converging visceral and somatic afferent pathways. *Am J Physiol Gastrointest Liver Physiol* 290: G451–G457, 2006.
42. **Letourneau R, Pang X, Sant GR, and Theoharides TC.** Intragranular activation of bladder mast cells and their association with nerve processes in interstitial cystitis. *Br J Urol* 77: 41–54, 1996.
43. **Malykhina AP, Qin C, Greenwood-Van Meerveld G, Foreman RD, Lupu E, and Akbarali HI.** Hyperexcitability of convergent colon and bladder dorsal root ganglion neurons after colonic inflammation: mechanism for pelvic organ cross-talk. *Neurogastroenterol Motil* 68: 938–948, 2006.
44. **Marshall JC.** Inflammation, coagulopathy, and the pathogenesis of multiple organ dysfunction syndrome. *Crit Care Med* 29, *Suppl*: S99–S106, 2001.
45. **Marshall JC.** Modeling MODS: what can be learned from animal models of the multiple-organ dysfunction syndrome? *Intensive Care Med* 31: 605–608, 2005.
46. **Mayer EA, Naliboff B, Lee O, Munakata J, and Chang L.** Review article: gender-related differences in functional gastrointestinal disorders. *Aliment Pharmacol Ther* 13, *Suppl* 2: 65–69, 1999.
47. **McEwen B.** Estrogen actions throughout the brain. *Recent Prog Horm Res* 57: 357–384, 2002.
48. **Miao FJ, Janig W, and Levine J.** Role of sympathetic postganglionic neurons in synovial plasma extravasation induced by bradykinin. *J Neurophysiol* 75: 715–724. 1996.
49. **Morrison JF.** The neural control of pelvic viscera. *Baillieres Clin Gastroenterol* 2: 63–84, 1988.
50. **Morrison TC, Dmitrieva N, Winnard KP, and Berkley KJ.** Opposing viscero-visceral effects of surgically-induced endometriosis and a control abdominal surgery on the rat bladder. *Fertil Steril.* In press.
51. **Pang X, Cotreau-Bibbo MM, Sant GR, and Theoharides TC.** Bladder mast cell expression of high affinity oestrogen receptors in patients with interstitial cystitis. *Br J Urol* 75: 154–161, 1995.
52. **Papka RE and Mowa CN.** Estrogen receptors in the spinal cord, sensory ganglia, and pelvic autonomic ganglia. *Int Rev Cytol* 231: 91–127, 2003.
53. **Papka RE, Williams S, Miller KE, Copelin T, and Puri P.** CNS location of uterine-related neurons revealed by trans-synaptic tracing with pseudorabies virus and their relation to estrogen receptor-immunoreactive neurons. *Neuroscience* 84: 935–952, 1998.
54. **Pezzone MA, Christianson JA, Liang R, Fraser MO, and Davis BM.** Dual innervation by primary afferents of pelvic visceral organs. [Online] Program No. 286.4. 2004 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience, 2004. http://sfn.scholarone.com/itin2004/index.html.
55. **Pezzone MA, Liang R, and Fraser MO.** A model of neural cross-talk and irritation in the pelvis: implications for the overlap of chronic pelvic pain disorders. *Gastroenterology* 128: 1953–1964, 2005.
56. **Powell-Boone T, Ness TJ, Cannon R, Lloyd LK, Weigent DA, and Fillingim RB.** Menstrual cycle affects bladder pain sensation in subjects with interstitial cystitis. *J Urol* 174: 1832–1836, 2005.
57. **Qin C and Foreman RD.** Viscerovisceral convergence of urinary bladder and colorectal inputs to lumbosacral spinal neurons in rats. *Neuroreport* 15: 467–471, 2004.
58. **Qin C, Malykhina AP, Akbarali HI, and Foreman RD.** Cross-organ sensitization of lumbosacral spinal neurons receiving urinary bladder input in rats with inflamed colon. *Gastroenterology* 129: 1967–1978, 2005.
59. **Rouzade-Dominguez ML, Miselis R, and Valentino RJ.** Central representation of bladder and colon revealed by dual transsynaptic tracing in the rat: substrates for pelvic visceral coordination. *Eur J Neurosci* 18: 3311–3324, 2003.
60. **Rouzade-Dominguez ML, Pernar L, Beck S, and Valentino RJ.** Convergent responses of Barrington's nucleus neurons to pelvic visceral stimuli in the rat: a juxtacellular labelling study. *Eur J Neurosci* 18: 3325–3334, 2003.
61. **Sann H and Pierau FK.** Efferent functions of C-fiber nociceptors. *Z Rheumatol* 57, *Suppl* 2: 8–13, 1998.
62. **Saria A and Lundberg JM.** Evans blue fluorescence: quantitative and morphological evaluation of vascular permeability in animal tissues. *J Neurosci Methods* 8: 41–49, 1983.
63. **Shafik A, Shafik I, and El-Sibai O.** Effect of vaginal distension on anorectal function: identification of the vagino-anorectal reflex. *Acta Obstet Gynecol Scand* 84: 225–229, 2005.
64. **Shughrue PJ, Lane MV, and Merchenthaler I.** Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. *J Comp Neurol* 388: 507–525, 1997.
65. **Stanford EJ, Koziol J, and Feng A.** The prevalence of interstitial cystitis, endometriosis, adhesions, and vulvar pain in women with chronic pelvic pain. *J Minim Invasive Gynecol* 12: 43–49, 2005.
66. **Su HC, Wharton J, Polak JM, Mulderry PK, Ghatei MA, Gibson SJ, Terenghi G, Morrison JF, Ballesta J, and Bloom SR.** Calcitonin gene-related peptide immunoreactivity in afferent neurons supplying the urinary tract: combined retrograde tracing and immunohistochemistry. *Neuroscience* 18: 727–747, 1986.
67. **Szolcsanyi J.** Forty years in capsaicin research for sensory pharmacology and physiology. *Neuropeptides* 38: 377–384, 2004.
68. **Tabatai M, Booth AM, and de Groat WC.** Morphological and electrophysiological properties of pelvic ganglion cells in the rat. *Brain Res* 382: 61–70, 1986.
69. **Traub RJ.** Evidence for thoracolumbar spinal cord processing of inflammatory, but not acute colonic pain. *Neuroreport* 11: 2113–2116, 2000.
70. **Ustinova EE, Fraser MO, and Pezzone MA.** Colonic irritation in the rat sensitizes urinary bladder afferents to mechanical and chemical stimuli: an afferent origin of pelvic organ cross-sensitization. *Am J Physiol Renal Physiol* 290: F1478–F1487, 2006.
71. **Vera PL, and Meyer-Siegler KL.** Inflammation of the rat prostate evokes release of macrophage migration inhibitory factor in the bladder: evidence for a viscerovisceral reflex. *J Urol* 172: 2440–2445, 2004.
72. **Vliagoftis H, Dimitriadou V, Boucher W, Rozniecki JJ, Correia I, Raam S, and Theoharides TC.** Estradiol augments while tamoxifen inhibits rat mast cell secretion. *Int Arch Allergy Immunol* 98: 398–409, 1992.
73. **Wall PD, Hubscher CH, and Berkley KJ.** Intraspinal modulation of neuronal responses to uterine and cervix stimulation in rat L1 and L6 dorsal horn. *Brain Res* 622: 71–78, 1993.
74. **Wesselmann U and Lai J.** Mechanisms of referred visceral pain: uterine inflammation in the adult virgin rat results in neurogenic plasma extravasation in the skin. *Pain* 73: 309–317, 1997.
75. **Wesselmann U, Czakanski PP, Affaitati G, and Giamberardino MA.** Uterine inflammation as a noxious visceral stimulus: behavioral characterization in the rat. *Neurosci Lett* 246: 73–76, 1998.
76. **Willis WD.** Dorsal root potentials and dorsal root reflexes: a double-edged sword. *Exp Brain Res* 124: 395–421, 1999.