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Stress Management Affects Outcomes in the Pathophysiology of an Endometriosis Model

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Abstract

We have previously shown detrimental effects of stress in an animal model of endometriosis. We now investigated whether the ability to control stress can affect disease parameters. Endometriosis was surgically induced in female Sprague-Dawley rats before exposing animals to a controllable (submerged platform) or uncontrollable (no platform) swim stress protocol. Corticosterone levels and fecal pellet numbers were measured as an indicator of stress. Uncontrollable stress increased the number and size of the endometriotic cysts. Rats receiving uncontrollable stress had higher anxiety than those exposed to controllable stress or no stress and higher corticosterone levels. Uncontrollable stressed rats had more colonic damage and uterine cell infiltration compared to no stress, while controllable stress rats showed less of an effect. Uncontrollable stress also increased both colonic and uterine motility. In summary, the level of stress controllability appears to modulate the behavior and pathophysiology of endometriosis and offers evidence for evaluating therapeutic interventions.

Keywords

endometriosis, rat, stress, corticosterone, controllable, corticotrophin releasing factor, animal model

Introduction

Endometriosis is an estrogen-dependent gynecological disorder defined as the presence of endometrium-like tissue outside the uterine cavity, primarily on the pelvic peritoneum and pelvic organs.[1] This condition is characterized by peritoneal inflammation and is associated with severe chronic pelvic pain, pain during intercourse (dyspareunia), painful periods (dysmenorrhea), and infertility.[2] The etiology of endometriosis is still unknown, but women with this disease report more stress, anxiety, pain, and negative impact on daily life activities than women who have other pain syndromes (eg, chronic migraines).[3,4] Independent of pain levels, patients with endometriosis had higher scores of psychoticism, introversion, and anxiety when compared to patients of other pain disorders.[5] Patients with endometriosis who catastrophize were more likely to report persistent pain after therapy, suggesting that biopsychosocial variables could play important roles in the perceived and reported severity of pain in this patient population.[6] Moreover, it has been suggested that chronic pain causes downregulation of hypothalamic–pituitary–adrenal (HPA) responses as shown by reports of lower mean salivary cortisol levels in women having dysmenorrhea and endometriosis.[7,8] In accordance with these human studies, our own investigations with the rat model of endometriosis showed that immunoreactivity of hippocampal corticosterone-releasing factor (CRF) was lower in animals with endometriosis compared to sham-operated animals, independent of stress.[9] We speculate that the decreased CRF in the endometriosis rats might reflect an adaptive response to the chronic symptoms and/or the hyperactivated inflammatory responses. Together, these data suggest that endometriosis is associated with suppressed HPA axis responses and alterations in the central processing of stressful factors, which may negatively impact pain-related outcomes.

We have previously shown detrimental effects of stress in the development of endometriosis and its associated inflammatory parameters in an animal model of endometriosis, including increased numbers of lesions developed and the average lesion grade as well as increased inflammation (higher levels of MPO and greater mast cell infiltration in colonic tissues).[9] These data provided the first experimental evidence supporting the

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involvement of stress-activated neuroinflammatory mechanisms in this chronic, painful disease. We now investigated whether the ability to control stress can affect the observed negative outcomes. It is widely accepted that psychological stress plays a substantial role in the exacerbation of many chronic, inflammatory, and painful conditions.[10] Chronic psychological stress negatively impacts the regulatory process of inflammatory responses and neuroendocrine pathways, which in turn could lead to disease.[11,12] Although limited, there is some evidence that psychological interventions can downregulate stress and increase measures of quality of life in patients with endometriosis.[13] We propose to expand this knowledge by dissecting the mechanisms that underlie the possible beneficial effects of stress management interventions using an animal model of endometriosis. Our main goal is to support the development of psychological, behavioral, and stress management interventions as part of a multidisciplinary preventive and clinical management for patients with this painful condition.

Materials and Methods

The experiments reported herein were performed in accordance with the principles described in the "Guide for the Care and Use of Laboratory Animals," Publication No. DHMS (NIH) 86-23.

Animal Model

Studies were performed with female Sprague-Dawley rats weighing 200 to 250 g (Southern Veterinary Service, PSM, Puerto Rico), with 12 to 14 animals per treatment group. Animals were randomly assigned to 1 of the 4 experimental groups: sham-no stress, endo-no stress, endo-stress uncontrollable, or endo-stress controllable.

All animals were maintained in a restricted-access room with controlled temperature (23°C) and a 12-hour light–dark cycle. Standard laboratory chow and tap water were provided ad libitum. The Animal Care and Use Committee at Ponce School of Medicine approved all experimental procedures involving animals. Animals were handled for 7 days (5 min/d/rat) prior to beginning the experiment in order to reduce experimenter-induced stress on the animal, and daily vaginal cytological smears were carried out for all rats to check their reproductive cyclicity (Figure 1A). Regular cycling was demonstrated by noting morphological changes in the different phases of the rat cycle (diestrus, proestrus, estrus, and metestrus) over several days. The experiments were carried out at the same time of day (early afternoon) to minimize the influence of circadian rhythms.

Induction of Endometriosis

Endometriosis was induced surgically under pentobarbital anesthesia, based on the method by Vernon and Wilson.[9,14,15] Briefly, the distal 2 cm of the right uterine horn were removed

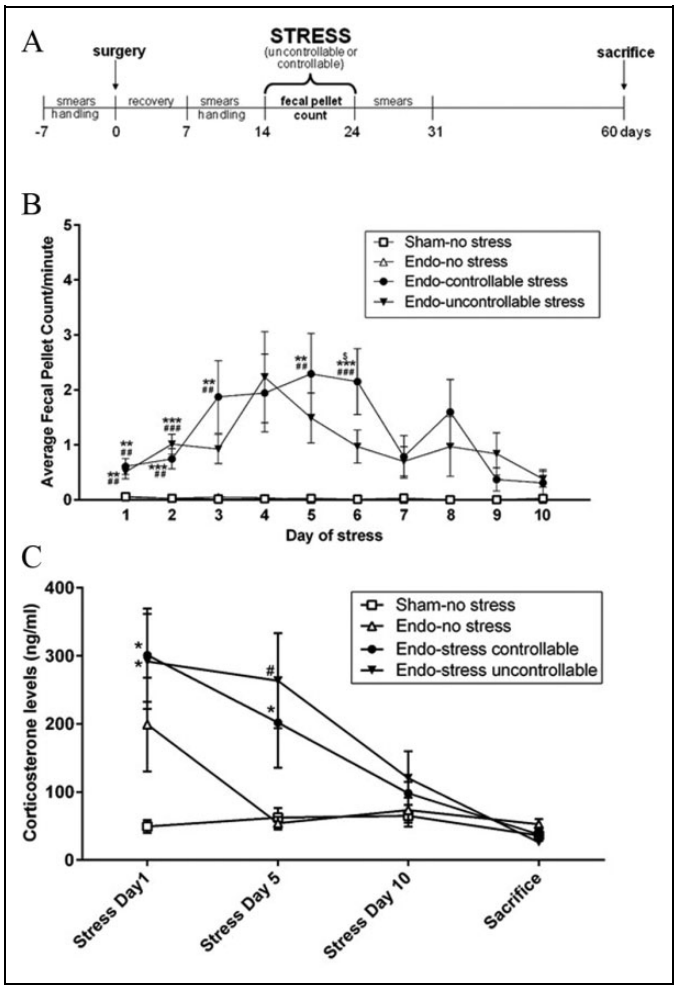

Figure 1. *Stress increases anxiety levels.* A, Animals were subjected to "controllable" (platform) or "uncontrollable" (no platform) swim stress for 10 consecutive days following the surgical induction of endometriosis. Endo-stress uncontrollable and endo-stress controllable groups had increased fecal pellet counts (B) and corticosterone levels (C), compared to the no stress groups, indicating increased anxiety levels (n = 12-14 ± SEM; $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ vs sham-no stress, $^{\#}P < .05$, $^{\#\#}P < .01$, $^{\#\#\#}P < .001$ vs endo-no stress, $^{\$}P < .05$ vs uncontrollable). SEM indicates standard error of the mean.

and immersed in warm (37°C) sterile culture medium. The endometrium was exposed by opening the uterine horn lengthwise with a pair of sterile scissors. Four pieces of uterine horn measuring 2 mm × 2 mm were cut. These implants were sutured with the serosal surface next to the mesenteric vessels of the small intestine and the endometrial surface exposed to the peritoneum. In the sham-operated group, 4 sutures were attached to the mesentery of the intestine without uterine implants, and the right uterine horn was massaged with fingertips for 2 minutes to minimize any effects resulting from the mechanical handling of the uterine horn. The peritoneal cavity was kept moist with copious amounts of saline solution throughout the surgery to reduce adhesions. Based on prior studies,[9,14,15] we allowed endometriosis to progress for 60 days following the induction surgery before killing.

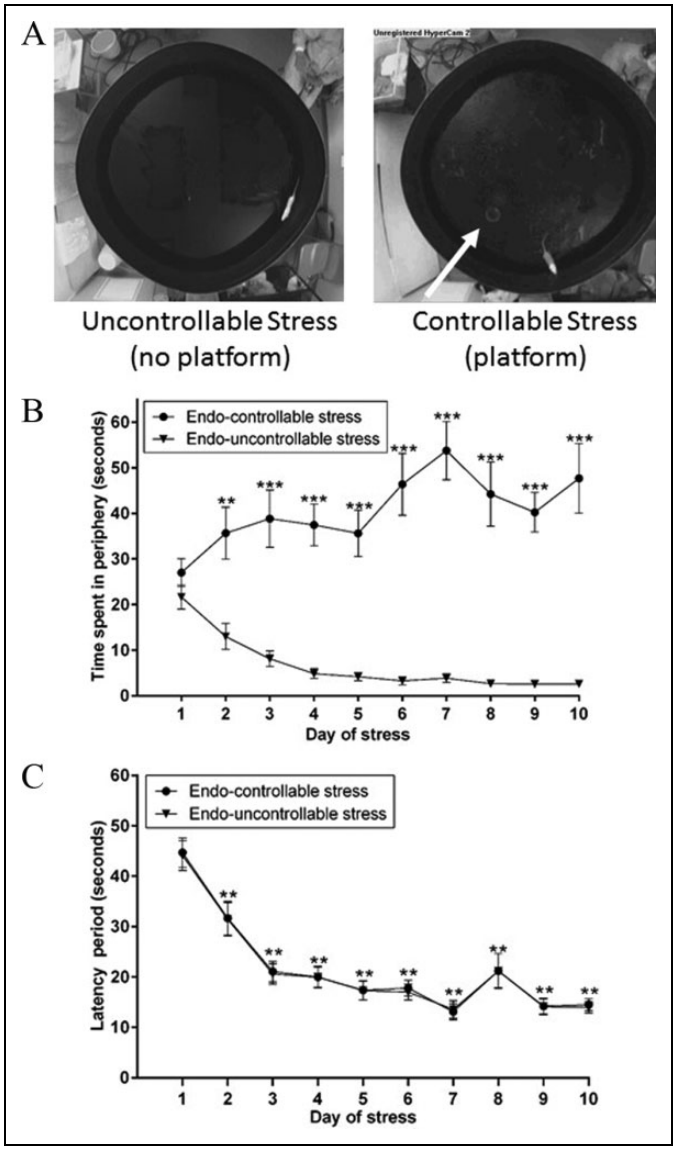

Figure 2. *The type of stress affects learning parameters.* A, Animals exposed to uncontrollable stress were allowed to swim freely whereas those exposed to controllable stress learnt to find a hidden platform. B, The endo-stress uncontrollable animals had more thigmotaxis activity than the endo-stress controllable group indicating increased anxiety (**$P < .01$, ***$P < .001$). C, There were no differences in the latency period (the time taken to find the platform) since animals were pair matched, and the animals learned to find the platform as demonstrated by significantly shorter latency periods compared to day 1 ($n = 14 \pm$ SEM; **$P < .01$ vs day 1). SEM indicates standard error of the mean.

Stress Protocol

To induce stress, animals were exposed to either an uncontrollable (no platform) or controllable (platform) swim stress protocol using a water maze apparatus 14 days after the surgical induction of endometriosis (Figure 2A).[16] The water maze apparatus consisted of a plastic pool (150 cm diameter and 60 cm deep) filled with water (37°C $\pm$ 1°C) and made opaque with nontoxic water-soluble paint. Salient extramaze cues were placed on the walls around the room. The position of the animals during the task was monitored and recorded using a ceiling-mounted video camera connected to a computerized tracking/imaging analyzer system (HVS-Image, Hampton, United Kingdom; Watermaze Software, Edinburgh, United Kingdom). Each animal received 10 trials of the stress protocol per day for 10 days. For each trial, animals were released into the water facing the edge of the pool from 1 of 4 equally spaced locations (N, S, E, and W) in a pseudorandom order and allowed to swim for a maximum of 60 seconds. Trials were separated by a 1-minute intertrial interval to allow the animal to rest. Each rat from the endo-stress controllable group was pair matched with a rat from the endo-stress uncontrollable group for the swimming time. The colonic propulsive activity was assessed by counting the number of fecal pellets expelled during each stress trial.[17,18] The average from the 10 trials was calculated for each day of the stress protocol and expressed as the number of fecal pellets/min. The sham-no stress and endo-no stress animals were transferred to clean cages for the equivalent amount of time and the fecal pellets expelled during the equivalent period of time were counted.

Measurement of Corticosterone Levels

Blood samples were obtained from the tail of the rats on days 1, 5, and 10 during the stress protocol and at the time of sacrifice. Serum corticosterone levels were determined using an ELISA kit (IBL-America, Minnesota) according to the manufacturer's instructions.

Collection of Tissues and Colonic Macroscopic Damage

At the time of killing, all animals had a cytological smear taken to allow for interpretation of any effects of the stage of estrous cycle on the experimental results. A laparotomy was performed to allow for assessment of disease severity as described subsequently and to collect tissues. The whole colon was removed and examined for macroscopic damage (presence of ulceration, presence of adhesions, colon thickness measured using a digital caliper, and presence of diarrhea) using an established, previously well-defined scoring system.[15] Tissue segments were fixed in 10% formalin, or weighed, frozen on liquid nitrogen, and then stored at −80°C until assayed.

Assessment of Endometriosis Severity

The peritoneal cavity was systematically examined for the presence of the implants and the original sutures. The classifications of vesicles in terms of grades of growth were carried out using a modification of a previously described scoring system.[9,15,19] In brief, the site of the implants was examined for the presence/development of vesicles or cysts and their longest length and width measured with a digital caliper. Vesicles <2 mm in length received a grade of 2, vesicles with fluid $\geq$ 2 mm and <4.5 mm received a grade of 3, vesicles $\geq$ 4.5 mm and <6.0 mm received a grade of 4,

and vesicles $\geq$ 6.0 mm received a grade of 5. If the implant had disappeared, it received a grade of 1.

Colonic Microscopic Damage

After routine processing, colon tissue segments of 4 μm were stained with hematoxylin and eosin to determine the extent of inflammatory infiltration and the appearance of the underlying muscle layers. Histological assessment of damage was performed using previously published criteria.[20] Briefly, the loss of mucosal architecture (0-3: absent, mild, to severe), muscle thickness (0 = muscle is less than 1/2 of mucosal thickness; 1 = muscle is 1/2 to 3/4 of mucosal thickness; 2 = muscle is equal to mucosal thickness; and 3 = all muscle), neutrophil infiltration (0 = none; 1 = in muscularis mucosae; 2 = in lamina propria/villi; and 3 = in serosa), crypt abscess formation (0 = absent; 1 = present), and goblet cell depletion (0 = absent; 1 = present) were evaluated. The score of each variable was added to give a total microscopic damage score (maximum of 11).

Measurement of Neutrophil Infiltration

Tissue myeloperoxidase (MPO) activity was determined in colonic and uterine tissues as an index of granulocyte infiltration. Myeloperoxidase is an enzyme found within the azurophilic granules of neutrophils and other cells of myeloid origin. It has been demonstrated previously that these levels reflect the state of inflammation.[21] Approximately 100 mg of flash-frozen tissues that were collected from mid-colon and uterine horn were analyzed. A modification of the technique described by Bradley et al was used.[22]

Mast Cell Numbers

Colon, uterus, and vesicle sections fixed in 10% formalin were cut at 4-μm thickness with a microtome (Microm HM340, Microm International, Germany) and mounted on glass slides. Tissue sections were deparaffinized with xylene substitute and hydrated through descending grades of ethanol to distilled water. They were subsequently stained for 2.5 minutes in Toluidine blue, followed by dehydration through 95% and absolute alcohols then cleared in xylene and cover slipped.

For analysis of chymase by immunofluorescence, mast cells were stained by indirect immunofluorescence with an antichymase (ab2377, Abcam, Massachusettes) primary antibody, and a highly cross-absorbed goat antimouse AlexaFluor 488 (A11029, Life Technologies, New York) secondary antibody. Briefly, formalin-fixed, paraffin-embedded tissue sections were deparaffinized with xylene substitute, rehydrated with graded alcohols, boiled in citrate-EDTA for antigenic recuperation, and blocked with normal goat serum. Tissues were incubated with primary antibody (1:50) overnight at 4°C in a humidifying chamber and then incubated with secondary antibody (1:100) for 30 minutes at room temperature in a humidifying chamber. All tissues were counterstained with a nuclear dye, 4',6-diamidino-2-phenylindole. A tissue section on each slide was used as a negative control, receiving phosphate-buffered saline instead of primary antibody. Tissues were visualized with an Olympus BX-60 microscope equipped with an X-cite light source. For each slide, the negative tissue was visualized first in order to adjust exposure to compensate for autofluorescence and nonspecific fluorescence. The tissues receiving primary antibody were then analyzed and 3 representative high-powered fields (HPFs) were photographed using the 40× objective and Nikon NIS-Elements (Nikon, New York). Chymase-positive mast cells in each HPF were counted using the Cell Counter plugin for ImageJ (NIH, Maryland). The average of the 3 HPFs for each animal was then used to calculate the number of mast cells per animal tissue in each treatment group.

Motility Responses

Segments of rat colon and uterus were mounted in 10-mL tissue baths in oxygenated Kreb solution at 37°C as described previously.[15] We measured isometric contraction of longitudinal and circular muscle using 1.0 g as the resting force (9.8 mN). Contractile responses to cumulative doses of carbachol (10^{-8} to 10^{-3} mol/L); a muscarinic cholinergic agonist) were measured. The tissues were weighed and dessicated, calculating tissue cross-sectional area and force exerted (mN/cm^2).

Statistical Analysis

Data were analyzed using GraphPad Instat version 3.0 (GraphPad Software, San Diego, California). A $P < .05$ was considered to represent a statistically significant difference. The mean difference $\pm$ the standard error of the mean was used to assess the differences before and after exposure to stress and among treatment groups. In order to assess the statistical significance of the mean differences, a parametric 1-way analysis of variance was used for normally distributed variables, using the Bonferroni post hoc pairwise contrasts to account for the accumulation of type I error. A Mann-Whitney U rank test was used to analyze differences between vesicle grades using the vesicle-grade arithmetic mean and 95% confidence intervals. A nonparametric Kruskal-Wallis H test was used for not normally distributed variables, and the Mann-Whitney U test was used for the post hoc pairwise contrasts after taking into account the accumulation of type I error.

Results

Anxiety

The number of fecal pellets produced during both stress protocols was used as an indirect index of anxiety related to stress.[17,23-25] During the 10-day stress protocol, the fecal output of the sham-no stress or endo-no stress animals (placed in new clean cages) remained around 0. Animals receiving stress had significantly higher defecation and

significantly higher corticosterone levels than the no stress groups indicating increased anxiety levels (Figure 1B and C). There were no significant differences between the sham-no stress and endo-no stress groups in fecal pellet counts or in corticosterone levels on any day of the protocol.

Learning Parameters

Animals with endometriosis receiving controllable stress (endo-stress controllable) showed less thigmotaxis activity (a tendency to stay close to the walls of the water pool, considered a sign of anxiety) than those receiving uncontrollable stress (endo-stress uncontrollable; Figure 2B). This difference was statistically significant. These animals learned to find the platform as the trials progressed, and the latency (time taken to find the platform) was significantly decreased from day 2 onward in both groups with an overall decrease in latency at day 10 compared to earlier time points (Figure 2C). Since animals receiving the uncontrollable stress were pair matched for time with those in the controllable stress group and removed from the pool, there was no difference in latency time between the 2 groups. There was also no significant difference in swimming speed between the 2 groups (data not shown).

Vesicle Size

There was no difference in the body weight between the groups at the time of sacrifice. After the animals were sacrificed, classification of the vesicles was performed as described.[15] As expected, none of the sham animals developed vesicles at the suture sites. In contrast, all of the endometriosis rats developed vesicles in at least one of the implant sites. The endo-no stress animals developed a vesicle in 77% of their sutures. This was increased in both endo-stress uncontrollable (85.71%) and endo-stress controllable (83.93%). The largest vesicle-grade mean was found among those exposed to uncontrollable stress, with twice as many vesicles of a size larger than 4.5 mm (grade 4 or 5, Figure 3A) and a significant increase in vesicle area per animal (Figure 3B). Moreover, only those animals receiving uncontrollable stress developed vesicles with a grade of 5 (diameter more than 6 mm). The vesicle grade mean difference was statistically significant when compared to those exposed to controllable stress but not statistically significant compared to the endo-no stress group ($P = .576$).

Colonic Damage and Cell Infiltration

Segments of colons were assessed both macroscopically and microscopically for damage. Endo-stress uncontrollable animals had significantly more macroscopic damage than endo-no stress or sham-no stress, whereas damage in the endo-stress controllable animals was no higher than the no stress groups (Figure 4A). Similarly, the microscopic analysis

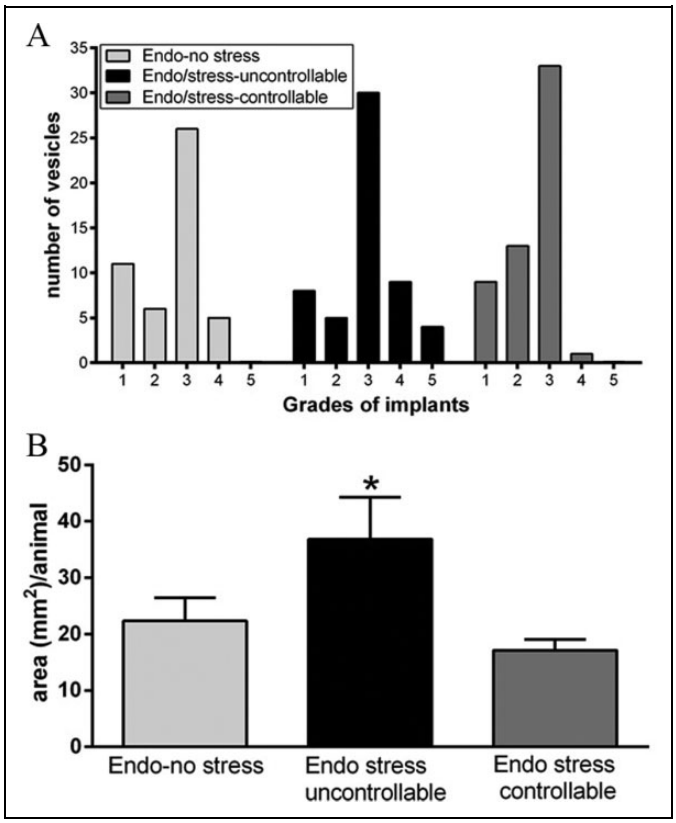

Figure 3. *Controllable stress decreases implant size.* None of the sham-no stress animals developed vesicles. The endo-no stress group (n = 12) developed a vesicle in 77% of their sutures (grades 2, 3 or 4). A, Exposure to uncontrollable stress increased the number of vehicles developed and their average grade; (B) animals exposed to uncontrollable stress (n = 14) had vesicles of a larger size than those found in the controllable group (n = 14); *$P < .05$ vs endo-stress controllable). SEM indicates standard error of the mean.

revealed more damage and cell infiltration in those animals receiving the uncontrollable stress (Figure 4B).

Samples of both colon and uterus were analyzed for measurement of myeloperoxidase activity to give an indication of neutrophil infiltration. The levels of myeloperoxidase were highest in the uteri of the endo-stress uncontrollable animals (15.92 $\pm$ 5.31 units/mg tissue) compared to 4.19 $\pm$ 1.09 units/mg tissue (sham-no stress), 9.82 $\pm$ 2.13 units/mg tissue (endo-no stress), and 10.18 $\pm$ 3.08 units/mg tissue (endo-stress controllable), but this difference did not reach statistical significance. No statistically significant changes were found between the groups for MPO levels in the colonic tissues (data not shown).

Colonic tissue from endo rats receiving stress contained more mast cells as assessed by toluidine blue staining (Figure 5A) with significantly increased chymase expression in the endo-stress uncontrollable group compared to no stressed groups and the endo-stress controllable group (Figure 5F). A similar result was observed in the vesicles (Figure 6A and B). No significant differences in uterine mast cell numbers were observed between the different groups (data not shown).

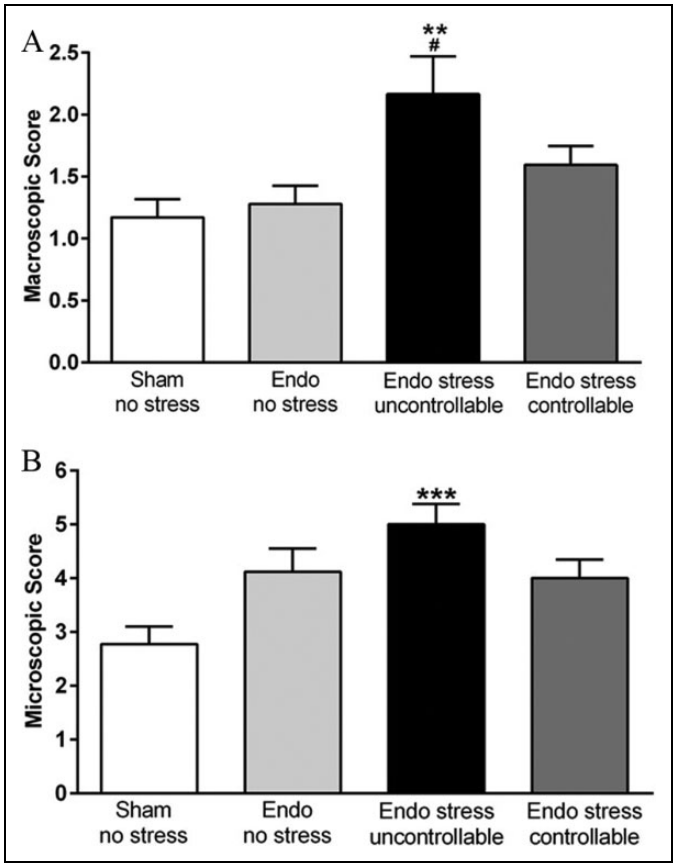

Figure 4. *Effect of stress on colonic damage.* Endometriosis animals receiving uncontrollable stress had (A) increased macroscopic damage (presence of adhesions, presence of diarrhea, thickness of the colon wall, and severity of ulceration) and (B) increased microscopic damage (loss of mucosal architecture, muscle thickness, neutrophil infiltration, crypt abscess formation, and goblet cell depletion) compared to animals receiving no stress. In contrast, damage in the endo-stress controllable group was no worse than that found in the no stress groups (n = 12-14 ± SEM; $^{**}P < .01$, $^{***}P < .001$ vs sham-no stress, $^{\#}P < .05$ vs endo-no stress). SEM indicates standard error of the mean.

Muscle Contractility

To investigate the possible impact of stress on the visceral hyperactivity associated with endometriosis, the motility of the colon and uterus was investigated in animals from the endo-no stress and endo-stress uncontrollable groups. Both longitudinal and circular muscle from the colon and uterus responded to carbachol in a concentration-dependent manner. However, the longitudinal and circular muscle from the uterus of animals receiving stress exhibited higher tension than did that of endo-no stress (Figure 7C and D). In the colonic tissue, only the longitudinal muscle of the endo-stress animals exhibited higher tension than that in the endo-no stress group (Figure 7A).

Discussion

In the present study, we demonstrate that the *degree and type of stress* are important for the exacerbation of endometriosis-associated signs and symptoms in the rat model. Our previous studies, which were the first to systematically investigate the effects of psychological stress on endometriosis, showed that stress prior to induction of endometriosis in rats exacerbates inflammatory parameters and that stress after the induction of endometriosis also exacerbates the condition.[9,26] Taken together, our data suggest that the level of stress controllability appears to modulate the presentation and pathophysiology of endometriosis and may thus offer possibilities for future therapeutic interventions based on stress-management strategies.

In this study, we observed that rats receiving uncontrollable stress had higher anxiety than those exposed to controllable stress or no stress. This was shown using various measures, including fecal pellet counts and corticosterone levels (measured during the stress protocol) and thigmotactic behavior, a well-accepted index of anxiety in rodents.[27] Interestingly, rats exposed to stress that was uncontrollable had more colonic damage and uterine cell infiltration, while in rats receiving controllable stress, there were less differences in these parameters. Stress controllability also appeared to modulate the presentation and pathophysiology of endometriosis: animals exposed to controllable stress had vesicles of a smaller average size than those found in the uncontrollable group, while exposure to uncontrollable stress increased the number of vesicles developed and their average severity grade. These data suggest that psychological stress impacts the growth of ectopically growing endometrium, and also the physiology of pelvic organs, and also augments local inflammatory parameters that can contribute to symptom exacerbation.

Stress is known to affect the physiology of organs such as the gut, leading to disorders such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS).[28] Not only does stress alter gut motility, but it is also known to increase perception of visceral pain.[10] Rodents exposed to a chronic stressor (repeated exposure to water avoidance stress) showed colonic hypermotility in a model of IBS.[24,29] Also, various rat models of stress show, among other signs of colonic dysfunction, visceral hypersensitivity.[30,31] In previous experiments, we have shown an increase in tension of the longitudinal muscle of the uterus in animals with endometriosis compared to normal controls.[15] This may be due to its direct exposure to the inflamed peritoneal environment and infiltration of inflammatory cells. In the present study, we expand on those findings and show that animals with endometriosis exposed to uncontrollable stress have increased motility in both colon and uterus, primarily in the longitudinal muscle, compared to rats with endometriosis not stressed. These contractility changes correlate with higher levels of cellular infiltration and of colonic damage in the colon. Increased numbers of mast cells were found in colonic tissue from both groups of rats exposed to stress, but the type of stressor had a significant effect on mast cell activation: the uncontrollable stress group had significantly more chymase staining compared to no stress and endo-

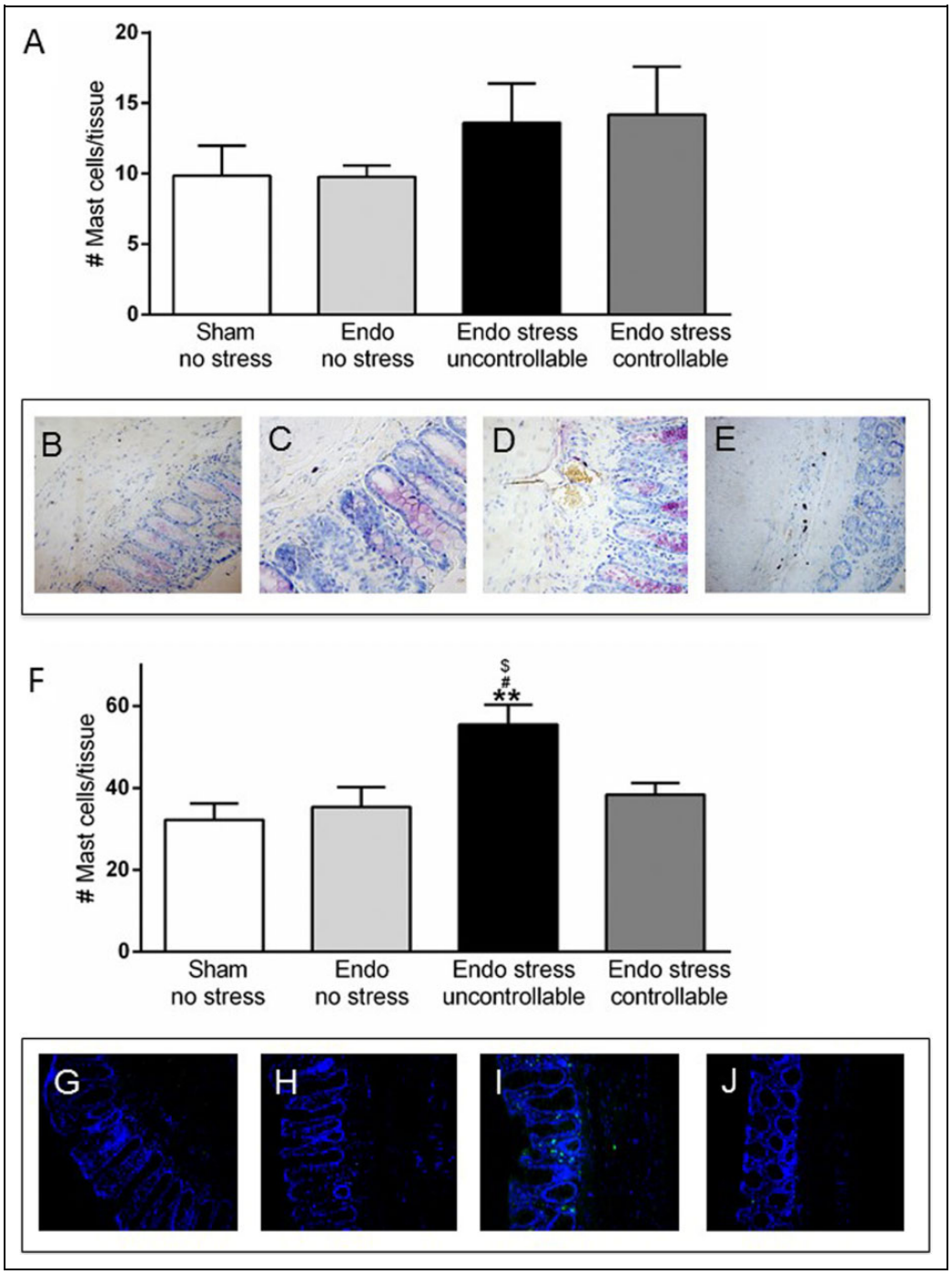

Figure 5. *Effect of stress controllability on mast cells in the colon*. A, Increased numbers of mast cells were found in colonic tissue from endo-stress rats (n = 10-14 ± SEM). Representative photos of Toluidine blue staining from colon in (B) sham-no stress, (C) endo-no stress, (D) endo-stress uncontrollable, and (E) endo-stress controllable. F, The uncontrollable stress group had significantly more chymase-positive mast cells compared to no stress groups and endo-stress controllable stress (**$P < .01$ vs sham, $^{\#}P < .05$ vs endo no stress, $^{\$}P < .05$ vs controllable stress group). Representative photos of (G) sham-no stress, (H) endo-no stress, (I) endo-stress uncontrollable, and (J) endo-stress controllable (40× magnification). SEM indicates standard error of the mean.

stress controllable groups. Mast cells are important modulators of stress signals via activation of the brain-gut axis, and their role promoting release of neurotransmitters and proinflammatory cytokines has to be taken into account when dissecting the mechanisms underlying the roles of stress in disease and painful symptoms specifically.[10] Our data thus suggest that stress can influence the colonic and uterine inflammatory parameters and contractility in animals with endometriosis, which may lead to the exacerbation of the gastrointestinal-related and painful symptoms of this disease.

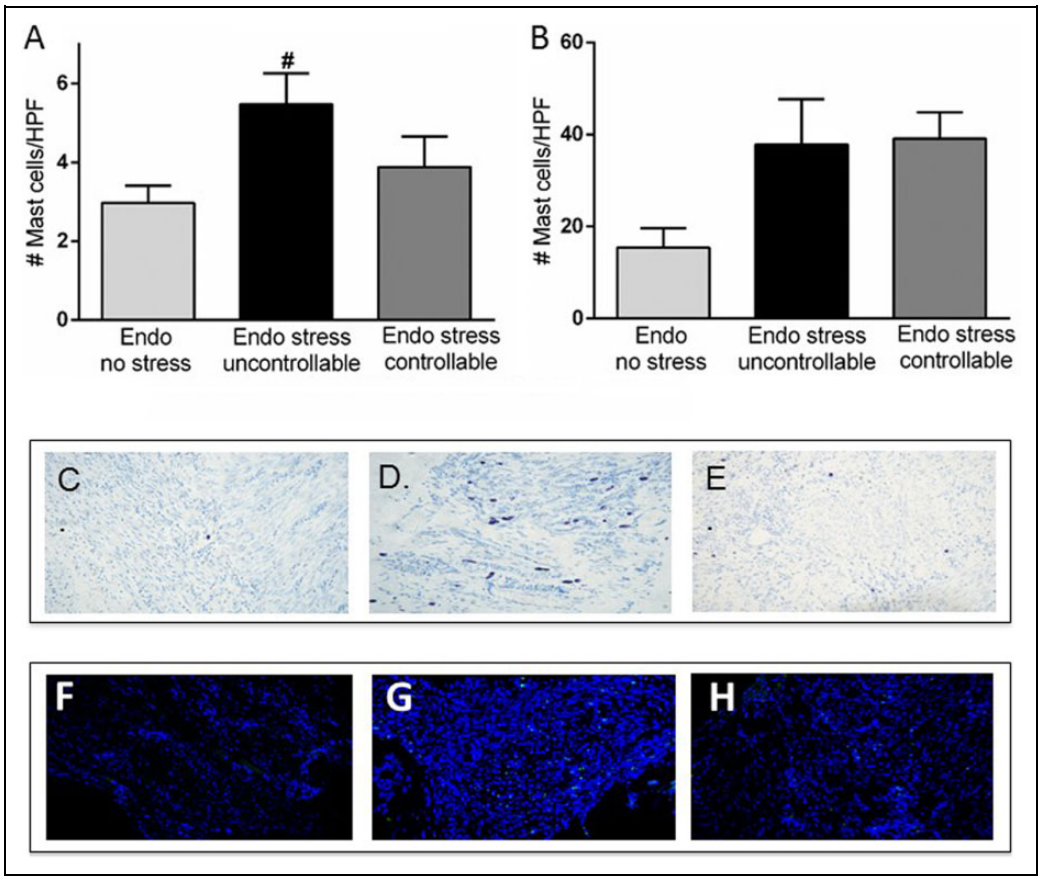

Figure 6. *Effect of stress controllability on mast cells in the vesicles.* A, Endometriosis animals exposed to uncontrollable stress had an increased number of mast cells as stained with Toluidine blue (n = 10-14 ± SEM; $^{\#}P < .05$ vs endo-stress controllable); (B) this pattern was echoed by chymase expression. Representative photos of Toluidine blue/chymase staining from vesicles in (C/F) endo-no stress, (D/G) endo-stress uncontrollable, and (E/H) endo-stress controllable animals (40× magnification). SEM indicates standard error of the mean.

It is still not clear how our findings might contribute to the etiology and/or the chronic pain associated with endometriosis, but higher uterine contractility could presumably result in increased levels of menstrual efflux into the peritoneal cavity and therefore higher risk for the development of endometriotic foci, as stated by the most accepted theory for the etiology of this disease, Sampson's theory.[32] Also, uterine hypercontractility plays an important role in many normal reproductive functions including menstruation, implantation, and parturition. When abnormal, it may be an important factor underlying reproductive disorders that include primary dysmenorrhea and endometriosis.[33,34] Of relevance to our study, high levels of uterine contractility have been shown to be associated with dysmenorrhea in women with symptomatic adenomyosis, in a context of high stress levels and pain.[35] Ongoing studies in our laboratories are investigating the effect of stress on the pain threshold and the influence of the brain-gut axis in these changes.

The swim stress paradigm has been used as a model for depression, anxiety, and other mood disorders in humans.[36,37] A modification of this protocol, the presence of a hidden platform in the water maze that rats can find and learn to locate on subsequent trials, models a situation where an individual is not totally hopeless in regard to their present stressful situation, thus the stress itself and its impact are controlled.[16,38] Our findings, therefore, have implications for the human scenario since significant correlations have been found between coping skills (eg, pain catastrophizing) and depression/anxiety,[39] which in turn can greatly impact the experience of pain. Depression in particular is known to decrease the threshold of pain reported.[40] Catastrophizing is a psychological trait that has been used as an index of pain sensitivity that can modulate clinical outcomes and pain perception.[41] The controllability of stress, among other factors, can minimize stressor-induced anxiety.[37] In sum, our data suggest that stress management strategies offer a target with great potential to alleviate painful symptoms and inflammatory parameters in endometriosis, an incurable disease that continues to substantially and negatively affect the quality of life of millions of women across the world.

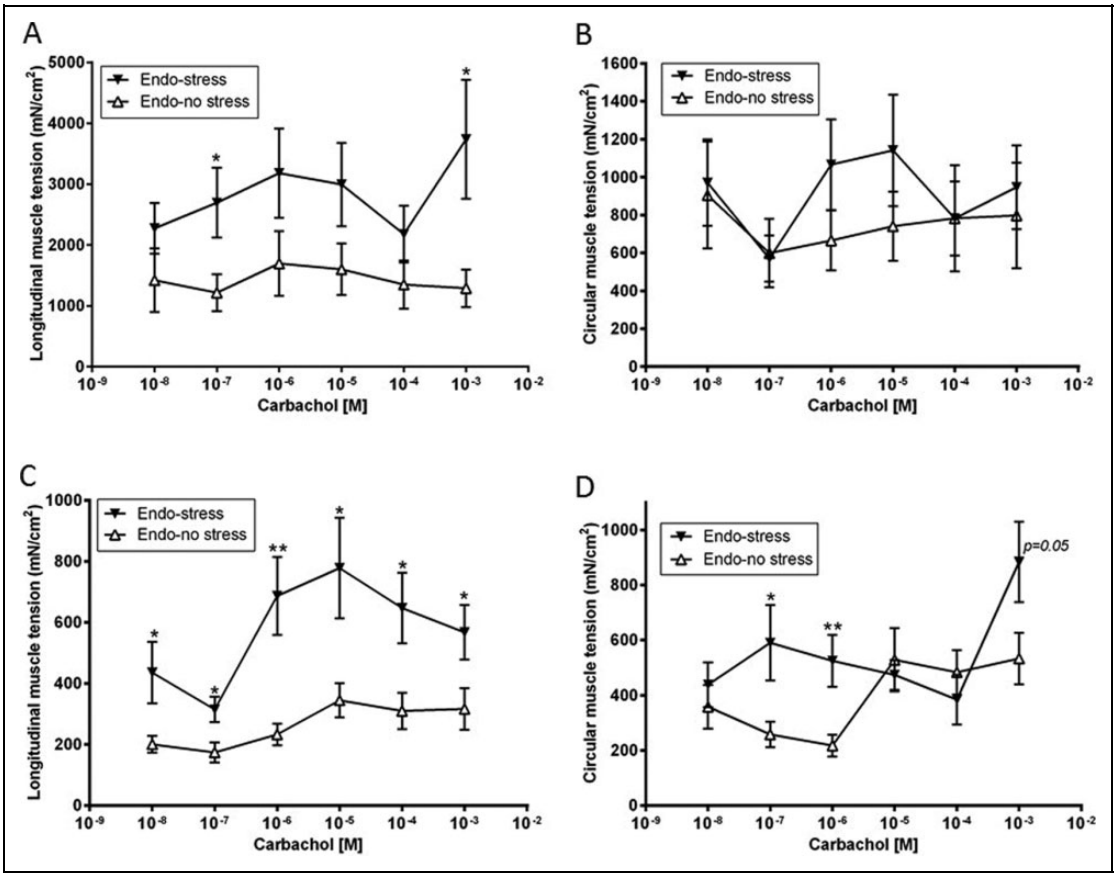

Figure 7. *Stress affects muscle contractility in the colon and uterus.* Both longitudinal and circular muscles from colon and uterus responded to carbachol in a concentration-dependent manner. A, The colonic longitudinal muscle of the endo-stress animals exhibited a significantly greater response to carbachol at 10^{-7} and 10^{-3} mol/L; (B) no differences in colonic circular muscle contractility were found between endo-stress and endo-no stress animals. C, The uterine longitudinal muscle of the endo-stress animals exhibited a significantly greater response to carbachol than did tissue from endo-no stress; (D) the circular muscle contractility of uterus from the endo-stress animals exhibited a significantly higher response at 10^{-7} and 10^{-6} mol/L carbachol (*$P < .05$, **$P < .01$ vs endo-no stress; n = 12-14 $\pm$ SEM). SEM indicates standard error of the mean.

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Authors' Note

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Declaration of Conflicting Interests

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