A New Model of Chronic Visceral Hypersensitivity in Adult Rats Induced by Colon Irritation During Postnatal Development

ELIE D. AL–CHAER, MOTOHIRO KAWASAKI, and PANKAJ J. PASRICHA
Division of Gastroenterology, Departments of Internal Medicine and Anatomy and Neurosciences, University of Texas Medical Branch, Galveston, Texas

Background & Aims: **The irritable bowel syndrome (IBS) is a common disorder characterized by abdominal pain in the setting of altered perception of viscerosensory stimuli. This so-called visceral hyperalgesia occurs in the absence of detectable organic disease in the peripheral organs and may cause normal or physiologic contractions to be perceived as painful. Although the pathogenesis of IBS remains speculative and is probably multifactorial, a prevailing paradigm is that transient noxious events lead to long-lasting sensitization of the neural pain circuit, despite complete resolution of the initiating event.** ***Methods:*** **Neonatal male Sprague–Dawley rats received either mechanical or chemical colonic irritation between postnatal days 8 and 21 and were tested when they became adults. The abdominal withdrawal reflex and the responses of viscerosensitive neurons were recorded during colon distention.** ***Results:*** **Colon irritation in neonates, but not in adults, results in chronic visceral hypersensitivity, with characteristics of allodynia and hyperalgesia, associated with central neuronal sensitization in the absence of identifiable peripheral pathology.** ***Conclusions:*** **These results concur largely with observations in patients with IBS, providing a new animal model to study IBS and validating a neurogenic component of functional abdominal pain that encourages novel approaches to health care and research.**

Pain, often viewed as a secondary and fleeting consequence of illness or injury, can become the consuming focus of a person's life when it is unremitting. This is particularly true when it occurs in the absence of any identifiable pathology, as in patients with irritable bowel syndrome (IBS), a condition that affects up to 15% of the U.S. population and has a significant socioeconomic impact.[1,2] IBS is presently defined by a bundle of symptoms[3] consisting mainly of recurrent abdominal pain associated with altered bowel movements. The original view of the disease as a primary disturbance in gut structure and/or function is being conceptually refined to include a complex and disordered interaction between the digestive and nervous systems.[4]

Although the relative roles of visceromotor, sensory, and affective factors[5] in the pathophysiology of this syndrome are not well understood, a specific enhancement of visceral (as opposed to somatic) sensitivity to noxious as well as physiologic stimuli appears to be consistently present and is considered a clinical hallmark of the syndrome.[1] The etiopathogenesis of this visceral hypersensitivity is probably multifactorial.[6] In this study, we propose that transient noxious stimulation of the viscera in neonates, in which the nervous system is most vulnerable, can cause long-lasting sensitization of the neural pain circuit despite complete resolution of the initiating event. We present a relatively novel approach to understanding the pathogenesis of IBS. In particular, this study shows for the first time that abnormal events in the gastrointestinal tract during postnatal development can have a long-lasting impact on the neural processing of sensory information in adulthood. It also shows that functional abdominal pain can be modeled in animals and makes the case for a neurogenic basis of this pain using direct behavioral and electrophysiologic evidence. Preliminary results were previously reported in abstract form.[7]

Materials and Methods

Animals

Experiments were performed using male Sprague–Dawley rats obtained as preweanling neonates (younger than 8 days) from Harlan Sprague–Dawley Inc. (Indianapolis, IN). Rats were housed in plastic cages containing corn chip bedding (Sani-Chips; PJ Murphy Forest Products, Montville, NJ) and maintained on a 12:12-hour light-dark cycle (lights on at 7 AM). The irritation procedure and the experimental testing were conducted during the light component of the cycle. The neonates were housed 12 in a cage with 1 adult female until

Abbreviations used in this paper: AWR, abdominal withdrawal reflex; CI, colon irritation; CRD, colorectal distention; IBS, irritable bowel syndrome.

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they were 25 days old. The adult female had access to food and water ad libitum. After separation, the male rats were housed 4 in a cage with access to food and water ad libitum. At the weight of 250 g, only 2 rats from the same testing group (control, mechanically irritated, or chemically irritated) were together in any cage. All studies were performed in accordance with the proposals of the Committee for Research and Ethical Issues of the International Association for the Study of Pain[8] and were approved by the Institutional Animal Care and Use Committee at the University of Texas Medical Branch in accordance with the guidelines provided by the National Institutes of Health.

Colon Irritation

Neonatal rats (8–21 days) were exposed to mechanical and chemical colon irritants for variable times and at different ages. The rats were then left to grow to adult age and tested for behavioral or neuronal responses to colorectal distention (CRD). Similar paradigms of colon irritation (CI) were used in periadolescent (older than 21 days) or young adult (older than 40 days) rats to help determine the existence of a critical age at which CI can result in chronic visceral hypersensitivity.

Neonatal CI. Male Sprague–Dawley rats (8 days old) were divided into 3 groups undergoing different treatments.

Rats in group 1 received CRD on a daily basis between the ages of 8 and 21 days. The distention was applied using angioplasty balloons (Advanced Polymers Inc., Salem, NH; length, 20.0 mm; diameter, 3.0 mm) inserted rectally into the descending colon. The balloon was distended with 0.3 mL of water, exerting a pressure of 60 mm Hg (as measured with a sphygmomanometer), for 1 minute and then deflated and withdrawn. The distention was repeated 2 times (separated by 30 minutes) within an hour.

Rats in group 2 received intracolonic injections of mustard oil (0.2 mL, 5%) daily between the ages of 8 and 21 days. Mustard oil was injected into the colon via PE90 tubing inserted to 2 cm from the anus.

Rats in group 3 (control) were handled similarly to those in groups 1 and 2 except that no colonic insertion was made. Rats in this group were gently held and touched on the perineal area daily between the ages of 8 and 21 days. Group 3 served as a control for CI and is referred to as the control group.

The rats in each group were housed in cages with their mothers. No treatment, procedure, or further intervention was done by the investigator for 2 weeks starting postnatal day 21 (PN21).

Periadolescent and adult CI. Initially, periadolescent (irritation began at PN21) and young adult (irritation began at PN45) rats were treated as 2 separate groups. However, when the rats were later tested with CRD, they showed no significant differences in behavior. Male Sprague–Dawley rats (older than 21 days), obtained from the same vendor, were divided into 3 groups similar to those described for neonatal CI. The adult rats were sedated with an intraperitoneal injection of Brevital (0.5 mL, 1%) to facilitate placement of the balloon or catheter into the descending colon and then allowed to wake up (Brevital, Jones Pharma, Inc., St. Louis, MO).

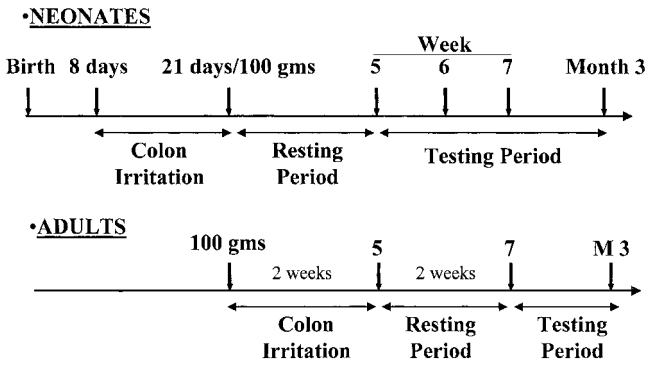

Figure 1. Timelines for CI and behavioral testing for neonatal (*upper line*) and adult (*lower line*) rats. CI began at age 8 days in neonates and 22 days in adults and continued for 2 weeks. It was followed by a resting period of 2 weeks. Rats were then tested on a weekly basis until age 3 months.

Rats in group 1 received daily CRD (80 mm Hg) using an inflatable balloon (constructed from a latex glove finger; length, 4 cm; inflated with air) attached to an intravenous line connected via a Y connector to a manual pump and a sphygmomanometer. The balloon was inserted into the colon while the animal was sedated, and CRD was induced while animals were fully awake. CRD was applied for 1 minute and repeated twice within an hour at an interval of 30 minutes.

Rats in group 2 received daily injections of mustard oil (0.5 mL, 5%) while sedated.

Rats in group 3 were sedated, allowed to wake up, and then gently handled by the investigator and touched in the perineal area. The schedule of procedures in adult groups was parallel in time to that in neonates (Figure 1).

Behavioral Testing

Behavioral responses to CRD were assessed in all groups starting 2 weeks after the cessation of the irritation protocol by measuring the abdominal withdrawal reflex (AWR) using a semiquantitative score or by measuring the threshold intensity of CRD that elicits an express contraction in the abdominal wall musculature. The AWR is an involuntary motor reflex similar to the visceromotor reflex.[9] However, the advantage of the AWR over the visceromotor reflex is that the latter requires additional surgery to implant recording electrodes and wires in the abdominal muscles, which may cause additional sensitization in an already sensitized system (CI rats). Distention balloons (described below) were placed in the descending colons of mildly sedated adult rats (Brevital, 5 mL, 1% intraperitoneally) and secured by taping the attached tubing to the rat's tail. The rats were then housed in small Lucite cubicles (20 × 8 × 8 cm) on an elevated Plexiglas platform and allowed to wake up and adapt (1 hour). Measurement of the AWR consisted of visual observation of the animal response to graded CRD (20, 40, 60, and 80 mm Hg) by blinded observers and assignment of an AWR score (AWR

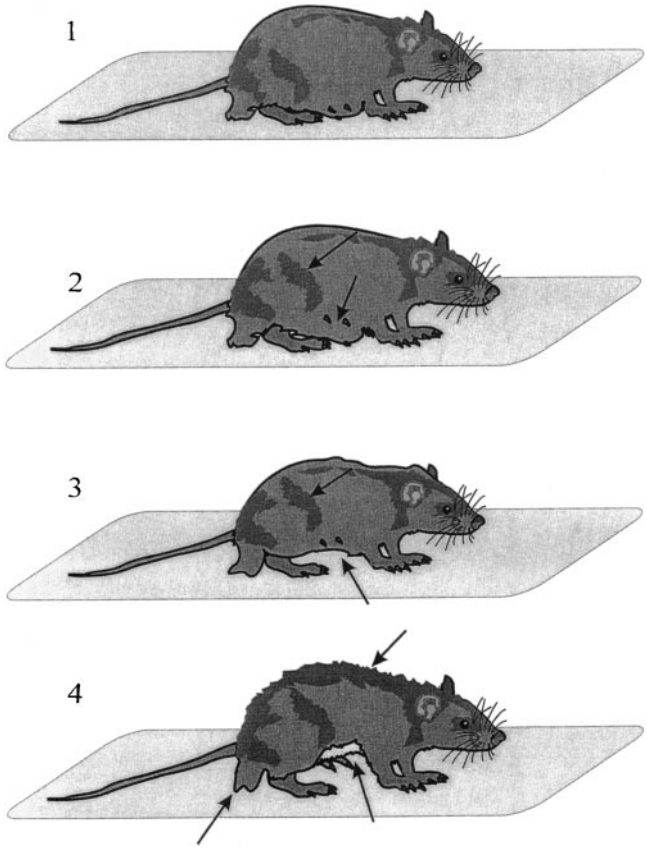

Figure 2. Schematic drawings of rats on similar platforms illustrating the behavioral scale based on visual observations of the AWR in response to graded CRD. (*1*) The rat becomes immobile during the CRD and occasionally clinches the head at the onset of the stimulus. (*2*) A mild contraction in the abdominal muscles is observed, but the rat does not lift its abdomen off the platform (*arrows* indicate the contraction observed in the abdomen and over the flank). (*3*) A strong contraction of the abdominal muscles is observed and the rat lifts its abdomen off the platform (*arrows* point to the lifting of the abdomen and the flank). (*4*) A severe contraction of the abdominal muscles is manifested by body arching and the rat lifts its pelvic structures off the platform (*arrows* point to the body arching, to the lifting of the abdomen and of the pelvic structures and scrotum).

scale; Figure 2): 0, no behavioral response to CRD; 1, brief head movement followed by immobility; 2, contraction of abdominal muscles; 3, lifting of abdomen; 4, body arching and lifting of pelvic structures.

Behavioral measurements were reproduced by 2 different blinded observers. Measuring the threshold intensity of CRD consisted of recording the stimulus intensity that evokes a visually identifiable contraction of the abdominal wall. CRD was applied in increments of 10 mm Hg starting at 10 mm Hg (the smallest distinguishable mark on the sphygmomanometer gauge).

For both measurements (AWR and thresholds), the rats were given CRD for 20 seconds every 4 minutes. To achieve an accurate measure, the distentions were repeated 5 times for each intensity. The data for each animal were averaged for analysis. The results obtained were compared across groups. A change in the magnitude or the threshold of the evoked response indicated a change in visceral pain processing. These results were compared among groups (neonatal CRD or mustard oil; control; adult CI). This comparison allowed us to determine whether a postnatal time line exists during which CI can lead to long-lasting visceral hypersensitivity.

Electrophysiological Preparations

Surgical procedures. Adult male rats of either neonatal groups (CI or control) were initially anesthetized with pentobarbital (60 mg/kg, intraperitoneally). The trachea was intubated and a catheter was inserted into one of the jugular veins to allow a continuous infusion of the anesthetic (5 mg $\cdot$ $kg^{-1} \cdot h^{-1}$). Body temperature was monitored and kept around 37°C by a servo-controlled heated blanket. A laminectomy exposed the L6–S1 segments of the spinal cord. The rat was then moved to a shielded recording table. The head of the rat was fixed in a stereotaxic instrument, and the vertebral column was mounted on a spinal holder to minimize movements. The dura mater was cut, and the exposed spinal cord was covered with warm mineral oil.

Neuronal recordings. Recordings were made by a blinded investigator from individual neurons in the spinal gray matter of control rats or rats with neonatal CI using tungsten microelectrodes (125 μm, 12 MΩ). The recording electrode was initially placed near the midline, moved mediolaterally at the level of 1 segment (on each side), then moved caudally spanning a depth of 1800 μm. This scheme minimized the damage to afferent input and allowed for observation of any lateralization of colonic input. Neuronal spikes were fed into a window discriminator and displayed on an oscilloscope screen. The output of both the window discriminator and the amplifier was led into a data collection system (CED 1401+; Cambridge Electronic Design, Cambridge, England) and a personal computer to compile rate histograms or wavemark files. Responses represent the total number of nerve impulses in excess of the number of background discharges that occur during the application of a stimulus. The measurements were compiled in a peristimulus time histogram. A period of 20 seconds of background activity was recorded, and a stimulus was applied. Cursors were set at the beginning and the end of the stimulus (generally a period of 10 or 20 seconds), and all the spikes that occurred between the cursors were summed. Cursors were also set at the beginning of the trace and after 10 seconds, and the spikes that occurred during this period were summed. This sum provides a measure of the background activity and is used to predict what the background activity would be during the stimulus. The 2 sums were divided by the respective duration, and the resulting averages (spikes/second) were subtracted to yield the value attributed to the response (total number of spikes/second in excess of the background activity during the stimulus).

Colon and somatic stimuli. Colon stimulation consisted of graded CRD produced by inflating a balloon inside the descending colon and rectum. The balloon was approximately 4 cm in length and was made of the finger of a latex

glove. It was attached to polyethylene tubing and inserted through the anus into the descending colon and rectum. The open end of the balloon was secured to the tubing with thread and wrapped with tape (1 cm wide). The balloon was inserted so that the thread was approximately 1 cm proximal to the anal sphincter. The balloon was held in place by taping the tubing to the tail. The tubing was attached via a T connector to a sphygmomanometer pump and gauge. Distention was produced by rapidly inflating the balloon to the desired pressure (20, 40, 60, or 80 mm Hg). Before they were used, the balloons were blown up and left overnight so the latex stretched and the balloons became compliant.

Somatic stimulation consisted of (1) innocuous brushing of an area of skin with repetitive strokes with a soft camel hair brush; (2) innocuous pressure on skin applied using a large arterial clip attached to a fold of skin (the clip exerts a pressure of 150 g/mm^2 and is not damaging or painful if applied to the skin of the experimenter); (3) noxious pinch using a small arterial clip applied to a fold of skin (the clip exerts a pressure of 550 g/mm^2 and is painful when applied to the skin of the experimenter; however, it does not produce overt damage to the skin); and (4) deep tissue stimulation using repetitive gentle pressures (2 seconds each for 10 seconds) applied against the body wall (body wall indentation) with the cotton end of a Q-tip.

Colon histology. At the end of the behavioral experiments, the distal 10 cm of the descending colon and rectum was removed, placed in 10% formalin, and sent for histologic processing. A cross section of the colon wall was fixed in formalin, dehydrated in graded alcohols and xylene, embedded in paraffin, and cut serially into 4 50-μm sections (8 μm apart) to be stained with H&E. Histology slides were scanned using a CoolSNA camera (RS Photometrics, Tucson, Arizona) mounted on an Olympus microscope (Olympus America, Melville, NY). Image analysis was performed using MetaMorph Imaging System (Universal Imaging Corp., West Chester, PA). The severity of lesions in the colon was graded as follows: 1+, mild (infiltration of a low number of neutrophils in the lamina propria and little or no interstitial edema); 2+, moderate (infiltration of moderate numbers of neutrophils in the lamina propria and moderate interstitial edema); and 3+, severe (diffuse infiltration of moderate to large numbers of neutrophils in the lamina propria and severe interstitial edema). The severity of inflammation in colonic mucosa (none, mild, moderate, or severe) was graded after a blinded review by our gastrointestinal pathologist.

Statistical Analysis

The statistical analysis was done using SigmaStat (Jandel, SPSS Science, Chicago, IL).

Behavioral results. A Friedman test was used to assess if scores changed across pressures within each group. The differences in the median values of the AWR score among the 3 treatment groups (groups 1, 2, and 3) at each pressure of CRD were compared using the Kruskal–Wallis 1-way analysis of variance (ANOVA) on ranks. If the Kruskal–Wallis test result was significant ($P < 0.05$), we performed pairwise comparisons using a Wilcoxon rank sum test with a Bonferroni correction at 0.05/3 to correct for multiple comparisons.

A 1-way ANOVA was performed to compare the differences in the median values of the thresholds to elicit a distinctive abdominal contraction measured in the 3 groups. This was followed by pairwise comparisons using a Bonferroni t test with a corrected P value of 0.05/3.

Stated significant results refer to a P value of $<0.05/3$.

Electrophysiological results. The differences in the median values of the neuronal responses to each intensity of CRD among the 3 treatment groups (groups 1, 2, and 3) were compared using the Kruskal–Wallis 1-way ANOVA on ranks. If the Kruskal–Wallis test result was significant ($P < 0.05$), we examined pairwise comparisons using the Dunn test with a Bonferroni correction at 0.05/3.

Stated significant results refer to a P value of $<0.05/3$.

Results

CI in neonatal rats leads to a state of chronic visceral hypersensitivity in adults manifested by increased contractility of abdominal muscles and hyperexcitability of viscerosensitive neurons in the lumbosacral cord. This hypersensitivity occurs in the absence of identifiable histopathology in the adult colon and does not alter the growth rate of the rats with CI. In fact, sections from the colons of 12 rats (4 with neonatal CRD, 4 with neonatal mustard oil treatment, and 4 controls) were examined. The tissues showed no significant structural damage or loss of crypts. Mucin depletion or increase in intraepithelial lymphocytes was not seen in any of the tissue examined. Slides from the 3 groups were rated as 1+.

Behavioral Study

Eight rats neonatally treated with repetitive colon distention (group 1) were tested with graded CRD as adults (ages 5, 6, and 7 weeks and 3 months). They showed a significant increase in AWR compared with control rats (Figure 3). The responses in group 1 were similar to those recorded in group 2 (treated neonatally with intracolonic injections of mustard oil). These changes (between CI groups and control) were significant for all intensities of CRD, indicating a shift in the dose-nexus between stimulus and response toward higher responses to lower-intensity stimuli.

The threshold to elicit a distinctive abdominal muscle contraction in response to CRD was measured in rats with neonatal CRD (n = 6; group 1), neonatal mustard oil–treated rats (n = 6; group 2), and control rats (n = 6; group 3). It decreased from 53.3 ± 2.8 mm Hg in the control group to 22.3 ± 3.0 mm Hg in the irritated groups.

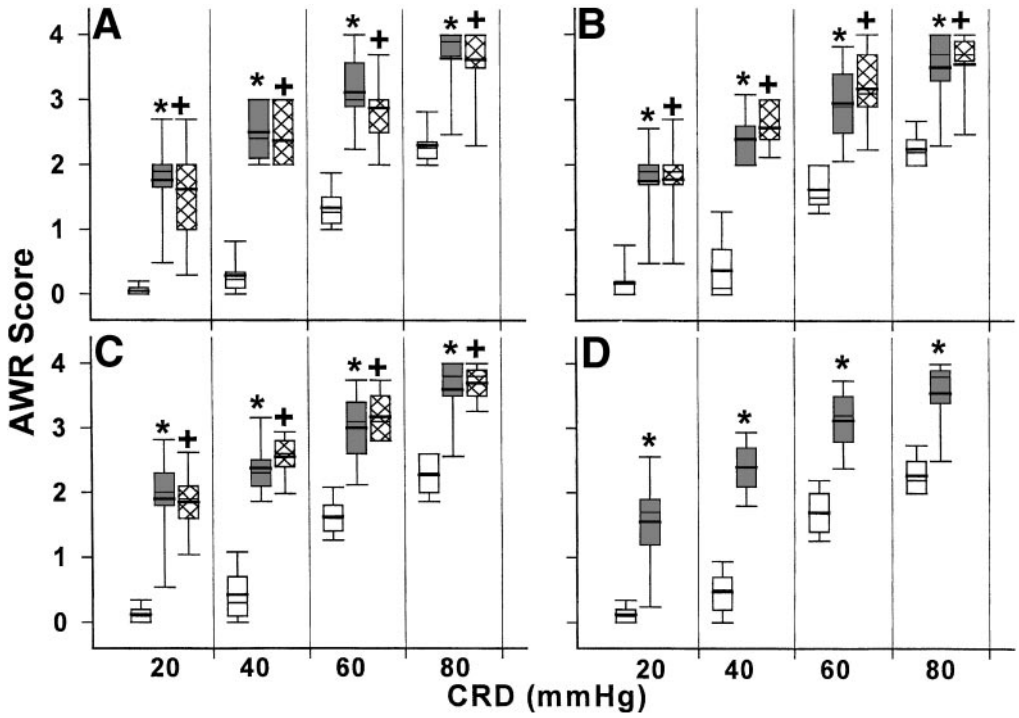

Figure 3. Box plots illustrating the distribution and mean AWR scores of 3 groups of neonatally treated rats measured at age (*A*) 5 weeks, (*B*) 6 weeks, (*C*) 7 weeks, and (*D*) 3 months: control (□; n = 8), rats with mechanical CI (■ ; n = 8; group 1), and rats that received intracolonic injections of mustard oil (⬚ ; n = 8; group 2). The AWR scores are measured in response to graded CRD (20, 40, 60, and 80 mm Hg). The vertical box plot displays the interquartile range (25% – 75%) and the median (*thick line*). The *error bars* define the 5th through 95th percentiles. Intracolonic pressure of <40 mm Hg would not be perceived as nociceptive in normal animals but becomes so in animals with neonatal CI; this phenomenon is typically referred to as visceral allodynia. *Significant difference between the responses of control and mechanically irritated rats (Bonferroni-corrected $P < 0.05/3$); $^{+}$significant difference in responses between control rats and rats neonatally treated with mustard oil (Bonferroni-corrected $P < 0.05/3$).

The behavioral responses of the rats that received CI after the age of PN21 (mechanical or chemical) were tested at the age of 7 weeks and 3 months. These responses did not differ significantly from those of the control groups.

Electrophysiological Study

Experiments were performed on 24 rats (8 with neonatal mechanical CI, 8 with neonatal chemical CI, and 8 controls). The responses of single viscerosensitive dorsal horn neurons located at L6–S1 spinal segments to CRD were recorded. Seventeen neurons were isolated and studied in rats with neonatal mechanical CI (group 1), 16 neurons in rats with neonatal chemical irritation (group 2), and 15 neurons in control rats (group 3, control). No significant differences were detected in either the baseline activity or the responses of neurons isolated in rats of the 2 groups with neonatal CI (groups 1 and 2). The baseline activity of the neurons ranged between 0 and 55 spikes/s for the rats in groups 1 and 2 and between 0 and 29 spikes/s for the control group. The average background firing rate (±SEM) of the neurons isolated from rats in group 1 or group 2 (25.0 ± 2.8 and 25.4 ± 3.7 spikes/s, respectively) was significantly higher than the average background activity of the neurons isolated in control rats (control; 7.0 ± 1.4 spikes/s; Figures 4*A* and 5*A*).

Individual responses of 2 neurons to graded CRD are shown in Figure 4*B*. Comparison of the average responses of the neurons isolated in rats of the 3 groups shows that the average response to CRD in rats with neonatal CI was significantly higher than the average response to CRD in control rats for all intensities tested (Figure 5*A*).

The neurons isolated in rats of all 3 groups and tested with CRD were also examined for convergent input from somatic receptive fields. The skin receptive fields were located in the perineal region, on the flank and upper thigh ipsilateral to the recording site, and at the base of the tail. Deep somatic fields often were located below the cutaneous receptive field. The propensity to respond to deep somatic stimulation was higher in rats with neonatal CI (both groups) than in control rats. The individual firing rates of these neurons are illustrated in Figure 4*C*. An extensive overlap, particularly in response to skin stimulation (innocuous brushing, innocuous pressure,

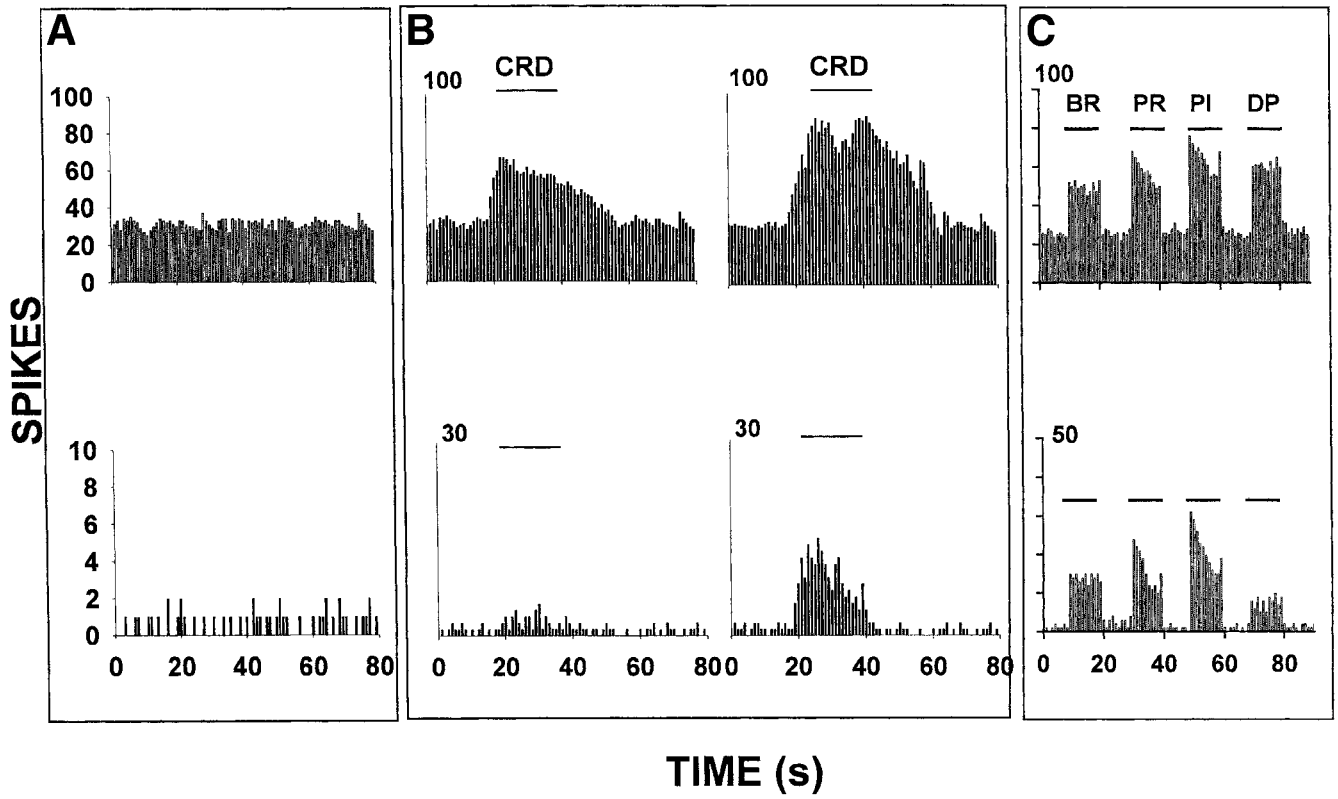

Figure 4. Rate histograms compiled from the responses of 2 neurons isolated in rats with neonatal CI (group 1; *upper row*) and control rats (group 3; *lower row*) show (*A*) the baseline activity of each neuron, (*B*) the responses of each neuron to 20 or 60 mm Hg (*CRD bar* indicates duration of distention), and (*C*) the responses of each neuron to all modalities of somatic stimulation (BR, brush; PR, press; PI, pinch and DEEP).

noxious pinch), was seen between the ranges of firing rates of neurons in rats of the 3 groups. On average, the differences in the mean values of the responses to brushing or pressing among the treatment groups were not great enough to exclude the possibility that the difference was caused by random sampling variability; there was not a statistically significant difference. However, there was a significant difference in the responses of neurons isolated in neonatally irritated rats (groups 1 and 2) and responses in control rats to pinching and deep tissue stimulation (Figure 5*B*), indicating the possibility of tenderness in the referred field to noxious cutaneous stimulation or deep probing of subcutaneous tissues.

Discussion

Pain is a central feature in the lives of patients with IBS; in the more severe cases, it can be prominent enough to give rise to the term "pain career."[10] The lack of overt visceral pathology, along with reported associations with childhood sexual, physical, and verbal abuse, has resulted in several psychologic hypotheses to explain this so-called functional abdominal pain.[6] Alternatively, such pain may be the result of sensitization of the nervous system at a vulnerable state in its development. The human body can be actively engaged in modulating the nociceptive input it is subjected to using multiple mechanisms to either increase or decrease the nociceptive input, the experience of pain, and the reflex responses to painful stimuli.[11–14] These mechanisms involve transient functional changes in peripheral and central neuronal activity and long-lasting changes involving neuronal plasticity.[15–18] Pain is normally evoked by stimuli that are sufficiently intense to activate high-threshold sensory fibers, which relay the signal to the spinal cord. However, tissue injury or inflammation may lead to profoundly increased pain sensitivity in which noxious stimuli generate a greater response (hyperalgesia) and stimuli that are normally innocuous elicit pain (allodynia). Hypersensitivity induced by repeated or persistent noxious stimuli is well documented. Clinically, it is a common observation that acute stimulation of the viscera or inflammation of some internal organs results in increased pain sensitivity.[19] For instance, distention of the colorectum in normal subjects evokes a painful sensation whose intensity increases progressively with the application of repetitive stimuli of constant amplitude.[20,21] However, the chronic visceral hypersensitivity observed in the absence of peripheral pathology, commonly described as

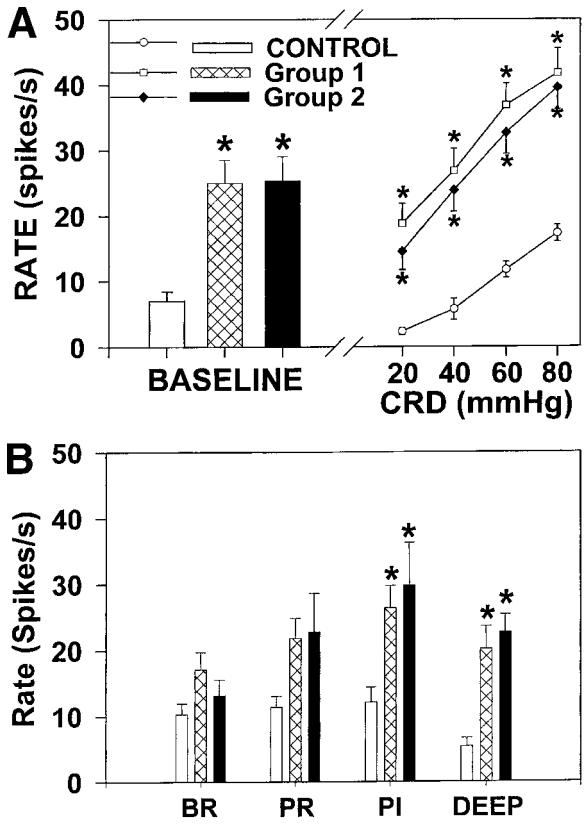

Figure 5. (*A*) Bar graphs illustrate the average background firing rate of visceroceptive neurons isolated in control rats (n = 15) and in rats with neonatal CI (group 1, n = 17; group 2, n = 16); line graphs illustrate the average responses to graded CRD (20, 40, 60, or 80 mm Hg) of the same viscereceptive dorsal horn neurons. (*B*) Bar graphs illustrate the average responses to different modalities of somatic stimulation of viscereceptive dorsal horn neurons isolated in the 3 rat groups (group 1, n = 17; group 2, n = 16; and control, n = 15). * Bonferroni-corrected $P < 0.05/3$.

functional abdominal pain, is poorly understood, in part because of the lack of a valid animal model.

The results of this study indicate that transient colonic irritation in the neonatal period can indeed result in chronic visceral hypersensitivity that persists through adulthood despite the absence of any identifiable histopathology. These observations concur with those usually made in patients with IBS, a syndrome characterized mainly by abdominal pain in the absence of peripheral injury. Furthermore, the visceral hypersensitivity seen in rats subjected to neonatal colonic irritation is associated with increased sensitivity of dorsal horn neurons recipient of colonic input. Finally, CRD in adult rats does not result in chronic visceral hypersensitivity. This finding may be attributed to a residual effect of the barbiturate given to the adult rats at the time of irritation on the memory of the event. However, it is more likely to be explained by a specific vulnerability of the nervous system in the neonatal period manifested by long-term plasticity.

A New Animal Model for Chronic Visceral Pain

Among the criteria considered in developing models of pain in nonhuman animals, the most important are the noxious quality, the reproducibility and control of the stimulus, the quantification of the response, and the ability to use the model in nonanesthetized animals. Allied with these requirements are a number of ethical considerations, including limitations on the intensity, duration, and timing of the stimuli and the ability of the animal to exhibit an escape response.[22] Existing animal models of bowel pain have greatly enhanced our knowledge of the nociception and hyperalgesia associated with acute mechanical or inflammatory stimuli, induced by phasic or repetitive CRD,[9,20,23] or induced by injection into the colon of inflammatory chemicals such as glycerol,[24] acetic acid,[25] mustard oil,[26] or zymosan[27] that largely mimic the symptoms of inflammatory bowel diseases but not IBS. The lack of a valid animal model of IBS has hampered research into the neurologic aspects of the disease.

In this model, we use balloon distention of the colon as a visceral stimulus. In awake rats, CRD produces an increase in arterial blood pressure, an increase in heart rate, and a visceromotor response that consists of a contraction of the peritoneal (skeletal) musculature in response to CRD.[20] These contractions, as monitored by a blinded observer, are graded with stimulus intensity and presumably correlate with a gradual intensification of viscerosensory perception that ranges from the least to the most painful and therefore provide an operant behavioral paradigm to test the responses to CRD. The rating of low and high levels of CRD in this study is consistent with psychophysical and pseudoaffective studies on the responses to CRD[9,20] showing that the pain threshold for CRD is approximately 40 mm Hg. The assumption that high-intensity CRD is a noxious stimulus and low-intensity CRD is not follows behavioral studies in humans that have shown that CRD of intensity $\geq$40 mm Hg is painful.[20] In rats, Ness and Gebhart[9] characterized the responses to CRD as noxious or nonnoxious based on whether the CRD evoked a visceromotor response (mean threshold, 26.3 $\pm$ 1.4 mm Hg) or simply produced a relaxation of the anal sphincter (mean threshold, 13.2 $\pm$ 0.7 mm Hg). The threshold for nociception corresponded with the threshold to evoke a visceromotor response.[9] In this study, similar considerations were followed to define innocuous or noxious CRD; the mean threshold to elicit a distinctive abdom-

inal contraction or a visually detected visceromotor response was 53.3 ± 2.8 mm Hg in control rats (group 3) and 22.3 ± 3.0 mm Hg in rats with neonatal CI (groups 1 or 2). This indicates that in control rats, stimuli of subthreshold intensities (i.e., 20 or 40 mm Hg) are nonnoxious, whereas stimuli of suprathreshold intensities (i.e., 60 or 80 mm Hg) are noxious. The higher threshold to nociception compared with that observed by Ness and Gebhart[9] may be explained by the shorter balloon/tubing setup used in this study to allow more flexibility in the hind body of the rat and observation of a wider range of behaviors. Studies in humans indicate that pain is produced by distention of the gut at the lowest distending pressures when long continuous segments of gut are distended simultaneously.[28] Differentiating noxious from nonnoxious intensities of CRD is particularly relevant to the definition of visceral allodynia and hyperalgesia (discussed below).

To study the behavioral effect of colon distention, we used a semiquantitative scale to measure the AWR in response to CRD. The AWR is an involuntary motor reflex similar to the visceromotor reflex.[9] It involves a supraspinal loop and is quantified by assigning a numerical score to the graded contractions of the abdominal muscles. The AWR is reproducible and graded with the intensity of the stimulus; it can be used to measure changes in the processing of visceral input in the same rat before and after a manipulation. The advantage of the AWR over other tests such as the visceromotor reflex is that the latter requires additional surgery to implant recording electrodes and wires in the abdominal muscles, which may cause additional sensitization in an already sensitized system. In these studies, we used a range of CRD intensities (20, 40, 60, and 80 mm Hg).

Innocuous intensities of CRD did not elicit a visually detected abdominal muscle contraction in control rats but evoked a nociceptive response in CI rats, indicating visceral allodynia. The aggravation of the responses to noxious CRD in CI rats compared with sham rats indicates visceral hyperalgesia.

Visceral Hypersensitivity: Allodynia or Hyperalgesia?

Most models of postinjury hypersensitivity revolve around the notion of central sensitization, which causes spinal cord neurons to enhance their excitability, acquire lower thresholds, or increase their responsiveness to peripheral stimuli. Central sensitization was proposed by Hardy et al.[29] to explain the development and spread of hyperalgesia after injury to the skin. Similar findings have been reported in recent years in studies on human subjects and experimental animals using intradermal injections of capsaicin.[30,31] Central sensitization also accompanies painful joint inflammation.[32] Furthermore, a growing body of scientific evidence points to a role of central sensitization in the hypersensitivity associated with acute or inflammatory visceral pain.[33–35] Therefore, it seems reasonable to propose that the persistent colon hypersensitivity observed in IBS is associated with central neuronal sensitization, manifested by an increase in neuronal excitability at spinal and possibly supraspinal levels. In this study, we have shown that spinal viscerоceptive neurons in rats with neonatal CI show signs of sensitization. These manifest as a heightened baseline firing rate of these neurons, a decreased threshold to activation by CRD, and exaggerated responses to CRD and somatic stimulation (intense and deep) compared with homologous neurons isolated from control rats. Whereas heightened somatic sensitivity has seldom been reported in association with acute visceral stimuli[36] (contrast with other results[37–41]), in this model of chronic visceral hypersensitivity there is a significant intensification of the neuronal responses to deep somatic stimulation within the perineal area. Referral of sensation, particularly pain, from one tissue to another is a common clinical phenomenon in musculoskeletal and visceral pain disorders and can be caused by convergence of peripheral afferents from skin, muscle, and viscera onto common central neurons. Furthermore, there seems to be a clinical impression that functional gastrointestinal disorders typically overlap with fibromyalgia in the same patient, suggesting a common cause.[42] Further studies are needed to qualitatively define the nature of the heightened somatic responses in this model and the underlying mechanisms of somatic hypersensitivity.

The heightened visceral sensitivity of neonatally irritated rats has characteristics of both visceral allodynia, in which the response to an innocuous CRD is rendered equivalent to that of a noxious CRD, and visceral hyperalgesia, in which a noxious CRD can generate a greater response than normal. The term *visceral allodynia* is more appropriate in the context of a painless stimulus (innocuous stimulus or physiologic process) experienced as painful because of a preexisting condition (not necessarily at the site of stimulation), whereas the term *visceral hyperalgesia* is more appropriate in the context of unusually heightened sensitivity to normally painful stimuli. In psychophysical terms, hyperalgesia and allodynia are best understood as the consequences of a leftward shift that occurs in the curve relating stimulus intensity to pain sensation following peripheral injury.[43] This use of the nomenclature is in accordance with the recommendation of the International Association for the Study of Pain (IASP) on the description of the components of

hypersensitivity states.[44] Despite that, the scientific literature generated nowadays continues to be replete with inaccurate use of terminology particularly when it comes to the descriptors of visceral pain. Visceral hyperalgesia, for instance, has been commonly used in clinical settings to refer to visceral pain, often without distinction between pain caused by normal physiologic functions and exaggerated pain caused by normally painful procedures. However, the distinction between the phenomena is of great importance. Visceral pain is commonly present as a combination of several heterogeneous conditions resulting in multiple pain symptoms. These symptoms could be differentially mediated by sensitization of different types of visceral nociceptors,[45–48] by a "phenotypic switch" in a subpopulation of large afferent fibers,[49] or by central plastic changes manifested by increased excitability of viscerosensory neurons in the spinal cord and in supraspinal centers.[33,39–41,50] Thus, it is important to distinguish between visceral allodynia and visceral hyperalgesia because they may have different underlying neuronal mechanisms and may require different approaches to treatment.

Long-term Effect of Neonatal Injury

Significant development of nociceptive neural circuits occurs during early postnatal life. Painful stimuli are normally absent or limited during this critical developmental period. Thus, when the pathways develop in the absence of noxious stimulation, pain represents a unique sensory stimulus. In the newborn organism, transient noxious stimuli have been shown to result in permanent alterations in afferent pathways.[51–54] In human neonates, sensitization can be produced by repeated mechanical stimulation or heel lances and in consequence of circumcision or surgery.[51,53] The neonatal nervous system appears particularly vulnerable and susceptible to plastic changes. The newborn spinal cord is more excitable than that of the adult, and this has been associated with differences in the state of A-fiber–induced excitation (such that neonatal A-fibers can evoke excitatory synaptic processes normally restricted to C-fiber input in adults) as well as in the composition, concentration, and activity of *N*-methyl-D-aspartate receptors.[54] In the adult, increases in excitability of the spinal cord nociceptive neurons induced by a sensitizing stimulus can be caused by the release of certain transmitters acting on the *N*-methyl-D-aspartate receptors,[27,55–58] by the properties of the neuronal network activated by the stimuli, or both. Analysis of these changes, particularly the ones associated with persistent nociception, has generated a great deal of interest. It is currently believed that central plasticity plays a fundamental role in the generation and maintenance of hyperalgesic pain states, and therefore its manipulation has become a target for potential therapies.

Conclusion

These observations demonstrate that the persistent visceral pain observed in the absence of identifiable pathology can be modeled in animals. This approach has potential for defining the pathophysiology of the visceral allodynia and hyperalgesia in functional abdominal disorders and hence should suggest ways the pain state may be ameliorated.

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Address requests for reprints to: Elie D. Al-Chaer, Ph.D., Departments of Internal Medicine and Anatomy and Neurosciences, University of Texas Medical Branch, 301 University Boulevard, Galveston, Texas 77555-0632. e-mail: edalchae@utmb.edu; fax: (409) 747-3084.

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