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# Neonatal maternal separation alters stress-induced responses to viscerosomatic nociceptive stimuli in rat

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**Coutinho, S. V., P. M. Plotsky, M. Sablad, J. C. Miller,  
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Neonatal maternal separation alters stress-induced responses to viscerosomatic nociceptive stimuli in rat. *Am J Physiol Gastrointest Liver Physiol* 282: G307–G316, 2002. First published October 3, 2001; 10.1152/ajpgi.00240.2001.— This study investigated the combined effect of neonatal maternal separation and acute psychological stress on pain responses in adult rats. Long-Evans dams and their male pups were reared under two conditions: 1) 180 min daily maternal separation (MS180) on postnatal days 2–14 or 2) no handling or separation (NH). At 2 mo of age, visceromotor responses to graded intensities of phasic colorectal distension (10–80 mmHg) at baseline as well as following acute 60 min water avoidance stress (WA) were significantly higher in MS180 rats. Both groups showed similar stress-induced visceral hyperalgesia in the presence of naloxone (20 mg/kg ip). MS180 rats had smaller stress-induced cutaneous analgesia in the tail-flick test compared with NH rats, with a residual naloxone-resistant component. MS180 rats showed an enhanced fecal pellet output following WA or exposure to a novel environment. These data suggest that early life events predispose adult Long-Evans rats to develop visceral hyperalgesia, reduced somatic analgesia, and increased colonic motility in response to an acute psychological stressor, mimicking the cardinal features of irritable bowel syndrome.

irritable bowel syndrome; stress; analgesia; naloxone

IRRITABLE BOWEL SYNDROME (IBS) is a disorder characterized by chronic abdominal pain and discomfort associated with alterations in bowel habits in the absence of a demonstrable pathology (67). Although IBS is likely a heterogeneous disorder in terms of etiology and pathophysiology, alterations in bowel habits are likely related to alterations in autonomic regulation of the gut, whereas symptoms of abdominal pain and discomfort are thought to involve additional changes in the perception of visceral events, in the form of visceral hyperalgesia or allodynia (46, 48). Additionally, in IBS patients without a concurrent diagnosis of fibromyal-

gia, visceral hypersensitivity is associated with a normal or diminished somatic pain sensitivity to noxious stimuli (13).

Progress in the development of more effective therapies has been hampered due to the lack of animal models that mimic the key features of IBS, in particular the enhanced perception of visceral events. Many investigations have utilized acute inflammatory insults to the gut using agents such as glycerol, mustard oil, acetic acid, or zymosan to produce acute visceral hypersensitivity, thereby mimicking inflammatory bowel disorders. However, IBS is a chronic disorder characterized by the absence of inflammatory changes in the gut mucosa. More recently, several potential IBS models have been reported, all of which mimic certain aspects of the human syndrome (2, 15, 62, 72). They include an early life colon irritation model (2), an adult stress sensitization model (62), and an adult postinfection model in the rat (15) and in the mouse (72). Although all of these models have greater face validity for IBS than the older acute inflammation models, they mimic only some aspects of the pathophysiological and pathogenetic features of the human syndrome.

Despite the incomplete knowledge about the pathophysiological mechanisms underlying symptom generation in IBS, a variety of putative mechanisms have been proposed (see Refs. 47 and 48 for reviews). Different types of stressors appear to play key roles in the development of IBS as well as in the modulation and maintenance of the disease throughout life. For example, early life stressors associated with childhood neglect, abuse, loss of a parent, and life threatening situations during childhood have been shown to increase the risk of IBS development (32, 34, 44). It has recently been demonstrated that early life events in humans are associated with a long-term enhancement in stress responsiveness and corticotropin-releasing factor (CRF) hypersecretion in adults (4, 10, 28, 39).

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The pattern of altered physiological responses to psychological and visceral stressors reported in IBS patients is also consistent with such a model of enhanced responsiveness of central stress circuits (47).

The effect of neonatal adversity on stress responsiveness in the adult has been extensively characterized in animals (35, 38, 51, 60, 75). These studies support the concept that the quality of the early life environment, in particular the mother-offspring interaction, along with genetic factors plays a prominent role in biasing an organism's response to psychological stressors, thereby increasing the risk of maladaptive responses to stressors throughout life (24, 29, 30, 56). In the rat, neonatal stress in the form of moderate periods of maternal separation of newborn rats results in permanent changes in the central nervous system, accompanied by a compromised ability to restrain the synthesis and release of CRF in response to acute laboratory stressors (3, 24, 58, 59). Well-characterized changes in such inhibitory central systems include a persistent regional decrease in the expression of glucocorticoid receptors (43, 51), an increase in regional norepinephrine release with a downregulation of  $\alpha_2$ -receptors (7, 24, 31, 60, 76), and decreased benzodiazepine receptor and  $\gamma$ -aminobutyric acid type A receptor binding in limbic regions (11). These neurochemical changes are associated with enhanced fearfulness, increased hypothalamic-pituitary-adrenal responsiveness to stressors, and an increased risk of developing depression-like behaviors (12, 43, 50, 59).

In the current study, we used this well-characterized rodent model of enhanced stress responsiveness to assess alterations in stress-induced visceral pain modulation. Given the likely involvement of adverse early life events and subsequent stressful events in adult life in the pathogenesis and chronicity of IBS symptoms (49), we sought to determine whether neonatal stress in rats combined with acute psychological stressors in adult life could be used to develop an animal model for IBS with high face and construct validity. Specifically, we wanted to address the following questions related to the long-term effects of neonatal maternal separation: 1) does it alter the pattern of stress-induced modulation of viscerosomatic sensitivity analogous to the pattern seen in IBS patients? 2) Does it alter the pattern of defecation following psychological stress? 3) Are the observed alterations related to changes in endogenous pain inhibition? Parts of these data have previously been published in abstract form (17, 19).

## METHODS

### *Animals*

Primiparous timed-pregnant Long-Evans dams were obtained from Harlan (Indianapolis, IN) on gestational day 12. On arrival, dams were individually housed in standard polycarbonate (46 × 25 × 20 cm) cages containing 2.5 cm of wood chip bedding material. Beginning at 0800 on postnatal day (PND) 2, dams were removed from their maternity cages to adjacent cages of identical type. All pups were then removed from their maternity cages, and sex was determined by measurement of anogenital distance. Female pups were

culled, and standardized litters of either 8 or 10 male pups were randomly assembled for fostering. Rearing conditions have been previously described (11, 42, 59, 73). Briefly, the dams and their litters were then randomly assigned to one of two rearing conditions: 1) handling/maternal separation group, exposed to a 180-min period of daily maternal separation on PND 2–14 inclusive (MS180) or 2) nonhandled comparison group, not exposed to handling or maternal separation (NH).

Protocols involving manipulation of the pups were initiated at 0800 by removal of the dams from the maternity cage (when they are off the nest) and placing them into separate identical cages until the end of the manipulation. On removal of the dams, MS180 litters were removed as a group from the nest, weighed, and placed as a group in an isolation cage (15 × 15 cm) in an adjacent room. These isolation cages were lined with 3 cm of wood chip bedding material and were placed into a neonatal incubator set to maintain an ambient temperature of  $32.0 \pm 0.5^\circ\text{C}$ . Pups further thermoregulated by burrowing into the bedding material and by huddling. Previous results indicated that pup core body temperature (rectal) remains at  $\sim 36\text{--}37^\circ\text{C}$  for up to 6 h. At the conclusion of the designated daily separation period, pups were returned to their maternity cage and rolled in the soiled home cage bedding material before reuniting them with the dam. Approximately 50% of the soiled bedding material in all cages was replaced with clean bedding and mixed well no more than once a week. On PND 22, rats were weaned and litter housed until PND 30, at which time they were housed in same treatment pairs. Animals were maintained on a 12:12-h light-dark cycle (lights on at 0600) with free access to food and water. Further experiments aimed at assessing viscerosomatic sensitivity at baseline and following stress were performed beginning at 2 mo of age ( $\sim 250$  g). All experimental protocols were approved by the Institutional Animal Care and Use Committee at the Greater Los Angeles Veterans Affairs Healthcare System.

### *Surgery*

Adult male MS180 and NH rats were deeply anesthetized with pentobarbital sodium (45 mg/kg, Nembutal, Abbott Labs, North Chicago, IL) administered intraperitoneally. Electrodes (Teflon-coated stainless steel wire, AstraZeneca, Mölndal, Sweden) were stitched into the external oblique musculature, just superior to the inguinal ligament, for electromyographic (EMG) recording as previously described (18). Electrode leads were then tunneled subcutaneously and externalized laterally for future access. Wounds were closed in layers with 4-0 silk. Following surgery, rats were housed in pairs and allowed to recuperate for at least 7 days. Wounds were tested for tenderness to ensure complete recovery from surgery before testing.

### *Assessment of Viscerosomatic Sensitivity*

The visceral stimulus employed was distension of the descending colon and rectum using a method that has been previously described (57). Briefly, animals were lightly anesthetized with halothane, and a flexible latex balloon (6 cm) lubricated with Surgilube (E. Fougera, Melville, NY) was inserted intra-anally into the descending colon. The balloon was positioned such that its end was 1 cm proximal to the anus and was secured in place by taping the balloon catheter to the base of the tail. Animals were allowed to recover for  $\sim 30$  min, and colorectal distension (CRD) was initiated by opening a solenoid gate to a constant pressure air reservoir. The balloon pressure, which represents intracolonic pres-

sure, was continuously monitored online with the aid of a customized pressure control device (AstraZeneca R&D). CRD in awake rats results in a contraction of the abdominal and hindlimb musculature (i.e., a visceromotor reflex; See Ref. 57). The visceromotor response (VMR) to CRD was quantified by measuring EMG activity in the external oblique musculature. The EMG activity was amplified and filtered (10,000 $\times$ , 300–5,000 Hz; Bioengineering, AstraZeneca R&D). The distending pressure and EMG activity were digitized and processed using a program written in LabView. Each distension trial lasted 60 s, and EMG activity was recorded 20 s before CRD (baseline), 20 s during CRD, and 20 s after termination of CRD. During CRD, there was an increase in EMG activity that was time-locked with the stimulus. The EMG activity was rectified, and the increase in the area under the curve (AUC) of EMG amplitude (over baseline) was recorded as the response. Baseline responses to graded intensities of phasic CRD (10, 20, 40, 60, and 80 mmHg; 20 s duration; 4 min interstimulus interval) were obtained in MS180 as well as NH rats. The animals were then exposed to water avoidance stress (WA) for 1 h, and responses to phasic CRD were obtained again.

In separate experiments, baseline responses to four consecutive trials of prolonged, tonic CRD (60 mmHg, 10 min duration, 16-min interstimulus interval) were also quantified by visual inspection of contractions in a blinded manner.

Somatic pain sensitivity was assessed using the thermal nociceptive tail-flick reflex (21). Animals were placed in Plexiglas cylinders in which they were awake and loosely restrained. Following a 45-min acclimation period, the tail-flick latency (TFL) was quantified by exposing the ventral surface of the tail to radiant heat and recording the time taken to withdraw the tail from the noxious thermal stimulus. Routinely, four TFL values were obtained at 5-min intervals for each animal, and the mean value was designated as the baseline TFL. The TFL was recorded at 5-min intervals following exposure of the animals to WA (1 h). A 10-s cutoff latency was used to avoid tissue damage.

Although pain is a subjective experience that requires a verbal description, animal studies technically do not measure or examine pain per se. However, in this study pain and nociception are used interchangeably when describing responses of the animal to visceral and somatic stimuli at an intensity that would produce pain in a human.

## WA

The test apparatus consisted of a Plexiglas tank (25  $\times$  25  $\times$  45 cm) with a block (8  $\times$  8  $\times$  10 cm) affixed to the center of the floor. The tank was filled with fresh tap water (25°C) to within 1 cm of the top of the block. The animals were placed on the block for a period of 1 h as previously described (9). This well-characterized test represents a potent psychological stressor with large elevations of ACTH and corticosterone within 30 min (53).

## Measurement of Fecal Pellet Output and Food Consumption

Naïve MS180 and NH rats were weighed, transferred, and housed individually in cages with indirect bedding (wire mesh bottoms) with unlimited access to food (preweighed) and water. The amount of food consumed (g) during a 24-h period (0800–0800) was quantified daily for a period of 7 days. Colonic motor function was simultaneously assessed by measuring fecal pellet output as previously described (no. of fecal pellets/24 h; see Ref. 9). After the acclimation period of 7 days, rats were exposed to WA and fecal pellet output and food consumption during the following 24 h were quantified.

## Data Analysis and Statistics

The VMRs to CRD were analyzed by comparing the same group of animals before and after a given treatment (WA or drug) using repeated-measures ANOVA of the raw EMG data collected for each animal at each pressure. For clarity of presentation, the data were normalized as percentage of the control response to 80 mmHg before WA or drug. In addition, the overall effect of any given treatment was determined by calculating the AUC of the raw EMG amplitude response as a function of pressure before and after treatment for each animal. From these values, the percent change in AUC was calculated by taking the difference between the AUC before and after treatment, dividing by the AUC before treatment, and multiplying by 100. These data were analyzed by a one-sample *t*-test to determine whether the overall change was significant. Likewise, TFLs were converted to percent change in latency by taking the difference between the TFL before and after WA, dividing by the baseline TFL before WA, and multiplying by 100. The overall effect of any given treatment was determined by calculating the AUC of the response as a function of time.  $P < 0.05$  was considered statistically significant in all cases.

## RESULTS

Rats were closely monitored throughout the neonatal period continuing into adulthood. No gross changes were observed in either body weight gain or linear growth between MS180 and NH animals.

### Baseline Visceral Sensitivity

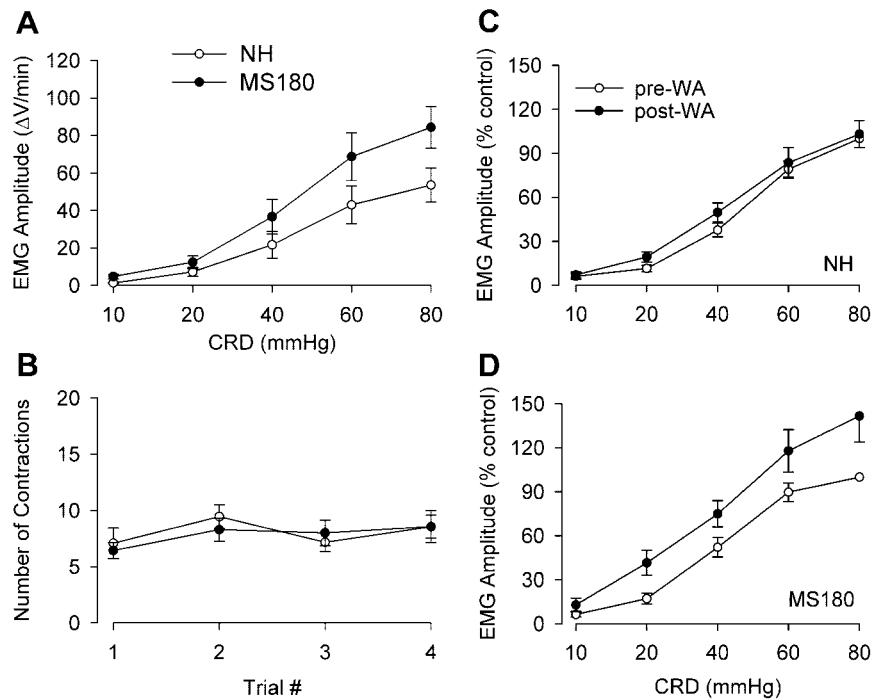
Baseline responses to graded intensities of phasic CRD were obtained in naïve MS180 ( $n = 27$ ) and NH rats ( $n = 13$ ; Fig. 1A). An inspection of the stimulus-response functions (SRFs) revealed a significant leftward shift in MS180 rats compared with NH rats ( $P < 0.05$ ). Response magnitudes were enhanced particularly at noxious intensities of CRD ( $>40$  mmHg).

Baseline responses to four consecutive trials of noxious, tonic CRD (60 mmHg) were also quantified in naïve MS180 and NH rats ( $n = 7$  each; Fig. 1B). In contrast to the difference in responses to phasic CRD, there were no statistically significant differences in responses to tonic CRD between MS180 and NH animals.

### Effect of Psychological Stress on Visceral Sensitivity

Responses to graded intensities of phasic CRD were recorded from MS180 and NH rats before and after acute psychological stress (WA, 1 h). These data are summarized in Fig. 1, C and D. Acute WA was without effect in NH rats ( $n = 9$ ; Fig. 1C); the two SRFs obtained before and after WA were virtually superimposable, and there was no significant change in the AUC after WA ( $0 \pm 18\%$ ). In contrast, in MS180 rats ( $n = 15$ ), acute WA resulted in a significant leftward shift of the SRF to phasic CRD ( $P < 0.007$  by repeated-measures ANOVA; Fig. 1D). Response magnitudes were significantly enhanced even at low, nonnoxious intensities of CRD, and this was reflected by a significant change in the AUC obtained after WA ( $57 \pm 13\%$ ;  $P < 0.001$ ). The possible confounding influence of re-

Fig. 1. Baseline and stress-induced visceral pain responses. **A:** visceromotor responses (VMR) to graded intensities of phasic colorectal distension (CRD) in nonhandled rats (NH;  $n = 15$ ) and rats separated from dams for 180 min/day (MS180;  $n = 27$ ). The responses were significantly different by repeated-measures ANOVA ( $P < 0.05$ ). Values are means  $\pm$  SE. **B:** VMR to 4 consecutive trials of noxious, tonic CRD (60 mmHg for 10 min) in NH ( $\circ$ ;  $n = 7$ ) and MS180 rats ( $\bullet$ ;  $n = 7$ ). Differences were not significant. **C:** VMR in NH rats ( $n = 9$ ) before and after water avoidance stress (WA; 60 min). There was no significant difference between conditions. In this and subsequent figures, values are means  $\pm$  SE calculated as %control response to 80 mmHg before WA. **D:** VMR in MS180 rats ( $n = 15$ ) before ( $\circ$ ) and after ( $\bullet$ ) WA.  $P < 0.007$ . EMG, electromyogram.



peated distensions on stress-induced visceral hyperalgesia was assessed in separate control experiments. Sham WA (1 h) was without effect in MS180 rats; i.e., the two SRFs were virtually identical (data not shown). Furthermore, control SRFs to graded intensities of phasic CRD were recorded from MS180 rats at 24-, 48-, and 72-h intervals; response magnitudes were found to be stable and virtually identical as previously reported (57).

#### Effect Size and Variability of Stress-Induced Visceral Hyperalgesia

To ascertain the interanimal variability in stress-induced visceral hyperalgesia in MS180 rats, the post-WA SRF was compared with the control, pre-WA SRF for all rats tested, including those that received vehicle for the studies describing the effect of naloxone on visceral nociceptive responses ( $n = 27$ ). The inset in Fig. 2 shows a representative example of EMG data as a function of CRD pressure for a single rat. AUC values were calculated before and after WA and expressed as the percent increase in AUC following WA. The magnitude of this increase represents stress-induced visceral hyperalgesia for each animal and is illustrated as a histogram of effect size in Fig. 2. Eighty-nine percent (24/27) of animals showed increases in the AUC following WA ranging between 15 and 100%; the mean increase in AUC following WA was  $57 \pm 13\%$ . In contrast, the mean change in AUC following WA in NH rats was  $10 \pm 10\%$ .

#### Effect of Naloxone on Visceral Nociceptive Responses

To determine the possible role of endogenous opioidergic systems in the enhanced VMR to CRD seen in MS180 rats, the nonselective opioid receptor antago-

nist naloxone was administered (20 mg/kg ip) before assessment of baseline responses and immediately following WA to MS180 as well as NH animals.

**Baseline responses.** Naloxone had no effect on the VMR of MS180 rats under baseline conditions ( $n = 6$ ; Fig. 3A) but caused a significant leftward shift of the SRF in NH rats ( $n = 6$ ;  $P < 0.007$ ; Fig. 3B), which was reflected by a significant change in the AUC ( $52 \pm 12\%$ ;  $P < 0.03$ ).

WA. Naloxone treatment exacerbated the WA-induced visceral hyperalgesia in MS180 rats ( $90 \pm 36\%$ ; Fig. 3C). Naloxone treatment also unmasked a significant ( $P < 0.006$ ) WA-induced visceral hyperalgesia in

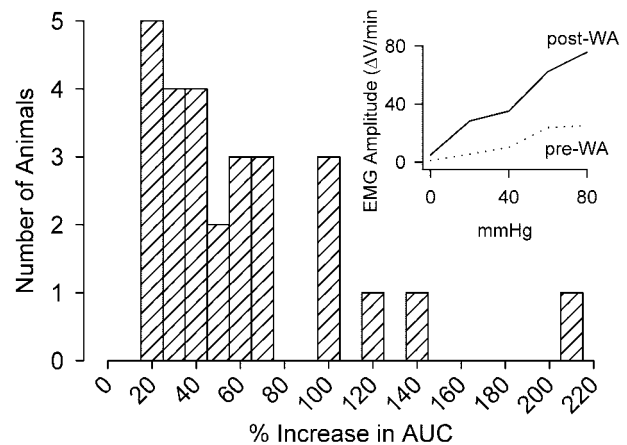


Fig. 2. Effect size of stress-induced visceral hyperalgesia in MS180 rats. Responses to graded intensities of phasic CRD were quantified before and after WA (inset shows representative experiment) in 27 MS180 rats. The overall effect of WA was determined by calculating the area under the curve (AUC) for the stimulus-response functions obtained before and after WA. Data are frequency histograms of the %increase in AUC following WA.

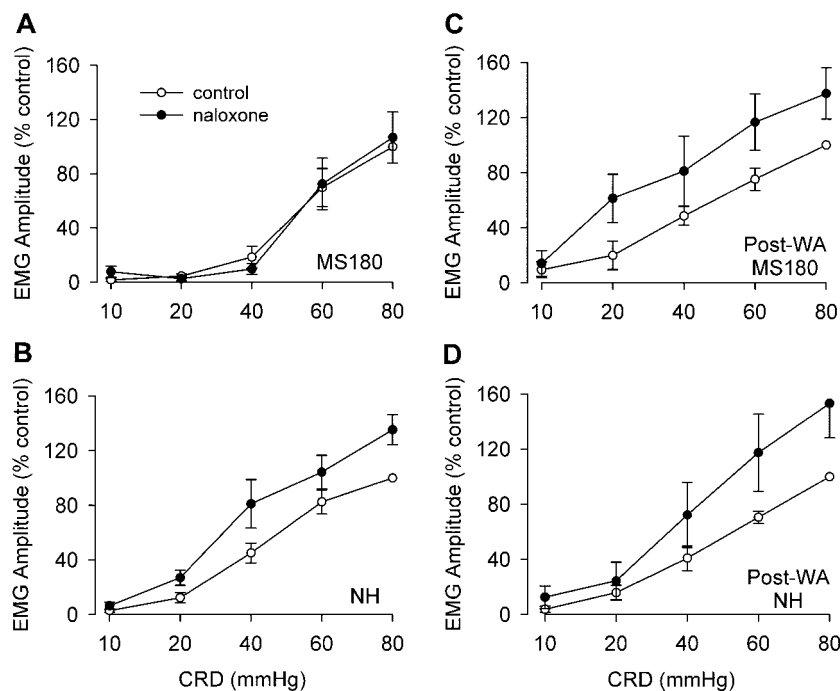


Fig. 3. Effect of naloxone on VMR to CRD. *A* and *B*: effect of naloxone (20 mg/kg ip) on MS180 (*A*) or NH rats (*B*). Responses to graded intensities of phasic CRD were quantified before and after drug or vehicle injection. Naloxone had no effect on VMR of MS180 rats ( $n = 6$ ) but produced significant visceral hyperalgesia in NH rats ( $n = 6$ ;  $P < 0.03$ ). *C* and *D*: stress-induced visceral hyperalgesia in MS180 (*C*) and NH rats (*D*). Responses to graded intensities of phasic CRD were quantified before and after WA. Naloxone (20 mg/kg ip) was administered immediately following WA. Naloxone treatment unmasked a significant hyperalgesia in NH rats ( $n = 8$ ;  $P < 0.01$ ) and exacerbated the effect in MS180 rats ( $n = 8$ ;  $P < 0.01$ ).

NH rats (Fig. 3*D*), which was similar in magnitude to that observed in control MS180 animals. This effect was mirrored by a large increase in the AUC ( $127 \pm 30\%$ ;  $P < 0.03$ ). There was no significant difference between SRFs of MS180 and NH rats obtained in the presence of naloxone following WA.

#### Baseline Cutaneous Sensitivity

Baseline cutaneous pain sensitivity was assessed in naïve MS180 and NH rats using the nociceptive tail-flick reflex. Individual baseline TFLs for all rats tested are illustrated in the *inset* in Fig. 4. The average baseline TFL for all MS180 rats tested was significantly longer compared with corresponding NH rats ( $3.76 \pm 0.18$  s vs.  $3.14 \pm 0.21$  s;  $n = 12$  and  $15$ , respectively,  $P < 0.04$ ).

#### Effect of Psychological Stress on Cutaneous Sensitivity

TFLs were quantified in MS180 and NH rats before and after acute WA and are illustrated in Fig. 4 as change in TFL relative to baseline. In contrast to its effect on responses to CRD, WA resulted in a significant cutaneous analgesia in NH rats ( $n = 5$ ;  $P < 0.001$ ). This effect was reversible, and TFLs reverted to near control levels by 60 min. Similarly, acute WA resulted in a significant and reversible cutaneous analgesia in MS180 rats ( $n = 4$ ;  $P < 0.001$ ). However, the magnitude of stress-induced analgesia was significantly diminished in MS180 rats compared with NH rats ( $P < 0.01$ ).

#### Effect of Naloxone on Stress-Induced Cutaneous Analgesia

To determine the possible role of endogenous opioidergic systems in stress-induced cutaneous analgesia,

naloxone was administered (20 mg/kg ip) immediately following WA to MS180 ( $n = 8$ ) as well as NH ( $n = 9$ ) animals. Naloxone treatment virtually abolished the stress-induced cutaneous analgesia in NH animals compared with saline-treated controls (Fig. 5*A*). In contrast, the stress-induced cutaneous analgesia was attenuated but significantly present in naloxone-treated MS180 rats compared with saline-treated controls ( $P < 0.005$ ; Fig. 5*B*). The time response AUC of

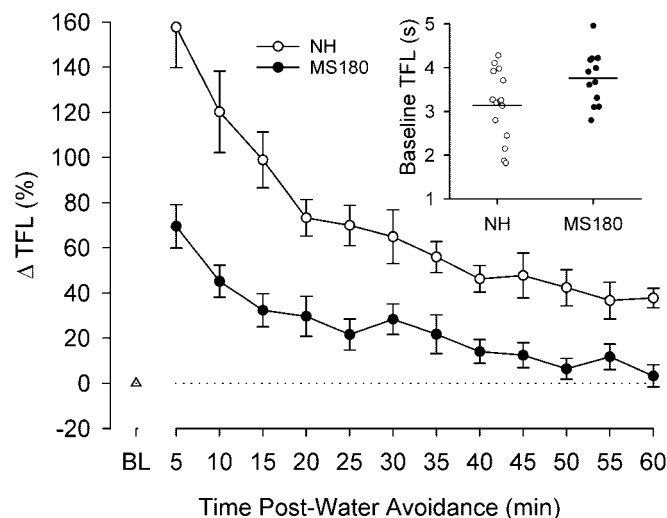


Fig. 4. Baseline and stress-modulated somatic pain responses. Tail-flick latencies (TFL) at baseline (*inset*) for 12 NH rats and 15 MS180 rats are shown. TFLs following WA are shown for NH ( $n = 5$ ) and MS180 ( $n = 4$ ). Although mean baseline TFLs were lower in NH rats ( $P < 0.04$ ), TFLs following WA were significantly higher in MS180 rats ( $P < 0.01$ ). Data are means  $\pm$  SE of the change in TFL over time following WA. Four values were obtained from each animal. The average baseline TFL for each animal tested is represented in seconds.  $\Delta$ , change in.

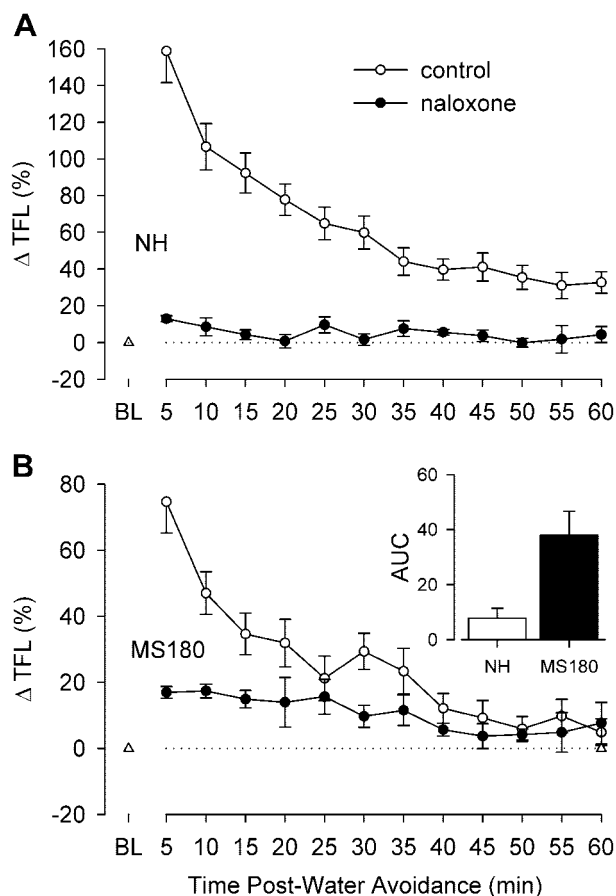


Fig. 5. Effect of naloxone on somatic pain responses. Effect of naloxone (20 mg/kg ip) on stress-induced cutaneous analgesia in NH rats (A;  $n = 9$ ) and MS180 rats (B;  $n = 8$ ) is shown. TFLs were quantified before and after WA. Naloxone (20 mg/kg) or vehicle (saline) was administered immediately following WA. Data are means  $\pm$  SE of the change in TFL over time following WA and naloxone/saline treatment. The overall effect of naloxone was determined by calculating AUC of the time-response curves (*inset*). Data are means  $\pm$  SE, calculated as %control response following saline administration.

naloxone-treated MS180 rats was 40% of the corresponding area of saline-treated MS180 rats (Fig. 5, *inset*).

#### Fecal Pellet Output and Food Consumption

In separate experiments, the effect of acute stress on colonic motor function was assessed by quantifying fecal pellet output in naïve age- and weight-matched MS180 and NH rats ( $n = 6$  each). Exposure to a novel environment (new cage) or acute WA resulted in a significantly enhanced fecal pellet output in MS180 rats compared with NH rats ( $36.0 \pm 2.1$  and  $37.1 \pm 1.2$  pellets/24 h for the MS180 rats vs.  $19.5 \pm 2.0$  and  $26.0 \pm 1.2$  pellets/24 h for the NH rats in the novel environment or following WA, respectively;  $P < 0.001$  for both comparisons). The increase in fecal pellet output was not accompanied by a concomitant change in food consumption ( $24.0 \pm 1.5$  and  $24.4 \pm 1.0$  g/24 h for the MS180 rats vs.  $25.9 \pm 0.9$  and  $24.3 \pm 1.1$  g/24 h for

the NH rats in the novel environment or following WA, respectively).

#### DISCUSSION

The present series of experiments demonstrates that neonatal stress in Long-Evans rats in the form of moderate maternal separation results in an altered adult phenotype with high face validity as an animal model for a subpopulation of IBS. The observed changes include mild visceral hyperalgesia and cutaneous hypoalgesia under baseline conditions, stress-induced visceral hyperalgesia, and enhanced stress-induced fecal pellet output. The high face validity of this model (alterations in visceral and somatic pain responses and autonomic responses) are complemented by an excellent construct validity (enhanced stress responsiveness) and a possible similarity in pathogenesis (adverse early life events) (32, 33, 36, 47).

#### Face Validity of the Model

**Visceral sensitivity.** Perceptual alterations in many IBS patients are characterized by a baseline hypersensitivity to phasic rectosigmoid distensions, particularly when delivered as an ascending series (52, 55). In contrast, baseline sensitivity to tonic distensions is either normal or decreased compared with normal subjects (40, 52). In the present study, compared with NH rats, nonstressed MS180 rats also showed a small but significant visceral hypersensitivity to an ascending series of phasic distensions, which was primarily seen in the noxious range, i.e., distension pressures  $>40$  mmHg (baseline visceral hyperalgesia). In contrast, and similar to the observations in IBS patients, no significant differences in the response to noxious tonic CRD were observed under control conditions. Additionally, MS180 exposed to WA, but not identically treated NH rats, exhibited significant stress-induced visceral hyperalgesia to graded intensities of phasic CRD. Physiological responses to WA have been well documented and are accompanied by central CRF release and elevations in plasma ACTH and corticosterone within 30 min (9, 45, 53). Evidence for acute stress-induced visceral hypersensitivity has been previously demonstrated in normal male Wistar rats following restraint stress (25) and following electrical foot shock (62, 64) and in Wistar-Kyoto rats (14). However, in the present study only MS180 animals developed stress-induced visceral hyperalgesia in adult life, consistent with a role for neonatal maternal separation in the manifestation of adult visceral hypersensitivity. Although stress-induced exacerbation of symptoms in IBS patients is a consistent finding, methodological problems preclude a definitive conclusion regarding stress-induced visceral hyperalgesia in humans (22, 23).

**Somatic hyposensitivity.** Several studies have demonstrated that IBS patients (without comorbid fibromyalgia) also show a normal or reduced sensitivity to noxious cutaneous stimuli, even when these are administered to the same dermatome as the rectosigmoid

(for example, see Ref. 13). Analogous to these findings, MS180 rats in the present study exhibited reduced baseline sensitivity to a noxious cutaneous thermal stimulus (applied to a different dermatome) compared with their NH counterparts, as evidenced by higher TFLs. Additionally, consistent with a large body of literature on stress-induced analgesia (see Ref. 8 for review), MS180 as well as NH rats exhibited significant stress-induced cutaneous analgesia following exposure to WA. However, the stress-induced cutaneous analgesia was significantly diminished in MS180 rats compared with NH rats, suggesting a compromised ability of MS180 rats to activate endogenous opioidergic pain inhibitory systems (see *Construct Validity of Model*). The naloxone-resistant component of stress-induced cutaneous analgesia in MS180 rats also suggests that compensatory analgesic mechanisms have likely developed in these animals. Given the hyperactivity of central noradrenergic systems in MS180 rats, it is possible that descending noradrenergic pain inhibitory pathways contribute to these compensatory mechanisms.

*Increased colonic motor response to stress.* Stress-induced increase in colonic motility has been well documented in rats (45, 65, 66). An increased responsiveness to CRF of pontine neurons projecting from Barrington's nucleus to the sacral parasympathetic nucleus and to the distal colon has been implicated as a central feature of functional pelvic visceral disorders, including IBS (71). In the current study, MS180 rats showed increased fecal pellet output in response to WA, as well as following exposure to a novel environment, compared with control NH animals. These findings are consistent with Valentino's model and parallel reports of increased sigmoid motility in response to emotional stressors in IBS patients (74).

#### *Construct Validity of Model*

*Role of opioidergic systems in mediating stress-induced modulation of viscerosomatic sensitivity.* In male rats, stress-induced somatic analgesia is mediated predominantly by descending opioidergic pain inhibitory pathways originating from the periaqueductal gray and the brainstem rostral ventromedial medulla (6, 27, 37, 54). Consistent with these findings, administration of naloxone virtually abolished the stress-induced cutaneous analgesia in control NH animals. In contrast, MS180 rats exhibited a significant residual stress-induced cutaneous analgesia following naloxone treatment.

The effect of naloxone on visceral pain responses also differed between the two groups. Naloxone enhanced the VMR to CRD in NH rats but not MS180 rats, consistent with a diminished pain inhibitory opioidergic tone in the latter group. Additionally, naloxone-treated NH animals exhibited significant stress-induced visceral hyperalgesia, resulting in a loss of group differences in stress-induced visceral hyperalgesia between NH and MS180 rats. These findings are consistent with a greater opioidergic pain inhibitory response

to stress in NH rats. Together, these data support the notion that activation of endogenous opioidergic pain inhibitory pathways attenuates baseline visceral pain sensitivity and prevents the development of stress-induced visceral hyperalgesia in control NH animals. Furthermore, compromised engagement of these systems appears to be involved in the development of stress-induced visceral hyperalgesia and diminished stress-induced cutaneous analgesia in MS180 animals.

Accumulating evidence suggests that the net effect of descending pain modulatory systems in models of cutaneous as well as visceral hyperalgesia is the result of simultaneously activated pain inhibitory and facilitatory mechanisms (20, 68–70). In the present study, it is possible that net descending pain facilitation as a result of compromised engagement of inhibitory opioidergic systems is a mechanism underlying the stress-induced visceral hyperalgesia and diminished stress-induced cutaneous analgesia in MS180 animals. In control NH animals, this facilitating influence is likely masked and overwhelmed by the simultaneous opioid-mediated pain inhibition, resulting in a lack of stress-induced visceral hyperalgesia.

Two observations in humans are consistent with an alteration in opioidergic systems. Consistent with the notion of compromised activation of central opioidergic systems, patients with functional dyspepsia, a syndrome closely related to IBS, have also recently been shown to have blunted central opioidergic tone (61). In addition, Lembo et al. (41) have recently demonstrated an enhanced visceranalgesic response to an exogenous  $\mu$ -opioid agonist (fentanyl) in IBS patients compared with healthy controls, suggestive of a possible upregulation of central  $\mu$ -opioid receptors involved in visceral pain modulation. When viewed together, these findings could suggest a compromised release of endogenous opioids in response to stressors both in the current animal model and in patients with functional gastrointestinal disorders.

Although untested in this model, other possibilities underlying the stress-induced visceral hyperalgesia include alterations in dorsal column-mediated pain modulation (2) or sensitization of visceral afferent nerve terminals by autonomically mediated changes in target cells in the colon following acute stress, such as mast cell degranulation (25) or enterochromaffin cell activation (25, 26).

#### *Comparison to Other Animal Models of Prolonged Stress Sensitization*

Three other animal models with potential relevance for IBS have been reported (2, 5, 15, 62, 64, 72). Two of these models (2, 62, 64), as well as the model presented in the current report, share long-lasting stress sensitization as a key pathophysiological mechanism underlying altered colonic motility as well as colonic hyperalgesia. Using a validated rodent model of posttraumatic stress syndrome (63), Stam et al. demonstrated long-lasting alterations in colonic motility responses (64) and in regional stress-induced Fos expression in



the brain (63) as well as preliminary evidence for increased visceral afferent responses to colonic distension (62). Al-Chaer et al. (2) used chemical or mechanical colon irritation in the neonatal period to induce long-lasting colonic hyperalgesia associated with alterations in bowel habits (E. D. Al-Chaer, personal communication). In view of the findings of this study, it is conceivable that part of the long-lasting stress sensitization in Al-Chaer's model may be related to altered mother-pup interactions resulting from the manipulations of the pups. For example, since maternal licking of the anogenital area is a key component of mother-pup interaction, manipulations of this area by colonic catheterization or instillation of chemicals may result in altered licking behavior. In contrast to the findings in the current report, and in contrast to observations in IBS patients (1, 13, 16), Al-Chaer et al. (2) observed indirect evidence for both visceral and somatic hyperalgesia in the same dermatome, consistent with a role for long-lasting sensitization of dorsal horn neurons, which receive converging viscerosomatic input. For example, the authors observed neuronal sensitization in the responses to somatic stimuli applied within the receptive field of the neuron (2).

In summary, we have used a combination of neonatal stress and subsequent exposure to psychological stressors in adult life to develop an animal model of IBS that mimics the main features of the human syndrome. The results of the present study are also consistent with a role for alterations in central circuits mediating autonomic responses and pain modulatory responses following psychological stressors in animals exposed to adverse early life events. Such enhanced stress responsiveness is a plausible pathophysiological mechanism underlying the dominant symptoms in a large number of IBS patients.

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