Impaired adaptation of gastrointestinal motility following chronic stress in maternally separated rats

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Bülbül M, Babygirija R, Cerjak D, Yoshimoto S, Ludwig K, Takahashi T. Impaired adaptation of gastrointestinal motility following chronic stress in maternally separated rats. Am J Physiol Gastrointest Liver Physiol 302: G702–G711, 2012. First published January 12, 2012; doi:10.1152/ajpgi.00447.2011.—Exposure to early life stress causes increased stress responsiveness and permanent changes in the central nervous system. We recently showed that delayed gastric emptying (GE) and accelerated colonic transit (CT) in response to acute restraint stress (ARS) were completely restored following chronic homotypic stress (CHS) in rats via upregulation of hypothalamic oxytocin (OXT) expression. However, it is unknown whether early life stress affects hypothalamic OXT circuits and gastrointestinal motor function. Neonatal rats were subjected to maternal separation (MS) for 180 min/day for 2 wk. Anxiety-like behaviors were evaluated by the elevated-plus-maze test. GE and CT were measured under nonstressed (NS), ARS, and CHS conditions. Expression of corticotropin-releasing factor (CRF) and OXT in the paraventricular nucleus (PVN) of the hypothalamus was evaluated by real-time RT-PCR and immunohistochemistry. MS increased anxiety-like behaviors. ARS delayed GE and accelerated CT in control and MS rats. After CHS, delayed GE and accelerated CT were restored in control, but not MS, rats. CRF mRNA expression was significantly increased in response to ARS in control and MS rats. Increased CRF mRNA expression was still observed following CHS in MS, but not control, rats. In response to CHS, OXT mRNA expression was significantly increased in control, but not MS, rats. The number of OXT-immunoreactive cells was increased following CHS in the magnocellular part of the PVN in control, but not MS, rats. MS impairs the adaptation response of gastrointestinal motility following CHS. The mechanism of the impaired adaptation involves downregulation of OXT and upregulation of CRF in the hypothalamus in MS rats.

Acute restraint stress; colonic transit; chronic homotypic stress; corticotropin-releasing factor; gastric emptying; oxytocin

FUNCTIONAL GASTROINTESTINAL (GI) disorders include functional dyspepsia (FD) and irritable bowel syndrome (IBS). Pathogenesis of FD and IBS is highly associated with stress in humans (23, 57). GI dysmotility may develop as a result of the accumulation of continuous or repeated stress in some individuals, while others are able to adapt to a stressful environment without developing GI symptoms. Corticotropin-releasing factor (CRF) in the brain acts to influence motor function of the GI tract. Restraint stress has been used as a physical and psychogenic stress model in rodents (31). Acute restraint stress (ARS) stimulates CRF in the amygdala and paraventricular nucleus (PVN) of the hypothalamus, resulting in activation of the hypothalamus-pituitary-adrenal (HPA) axis (23). Central CRF plays a dominant role: it delays gastric emptying (GE) and accelerates colonic transit (CT) following ARS in rats (34, 41, 71).

Oxytocin (OXT) is a cyclic nonapeptide hormone primarily synthesized in the magnocellular and parvocellular neurons of the PVN and supraoptic nucleus and secreted into the peripheral bloodstream from the posterior pituitary (2). Besides its well-defined physiological functions, such as milk ejection and induction of labor, OXT plays an important role in mediating stress responses. OXT is released from the neurohypophysial terminal into the bloodstream and within distinct brain regions in response to stressful stimuli. Central OXT has an anxiolytic effect and attenuates the HPA axis in response to stress in rodents (42). Acute or chronic central administration of OXT has been shown to reduce stress-induced corticosterone release and produce an anxiolytic effect in rodents (9, 62).

OXT administration significantly attenuates the release of ACTH and corticosterone and the increase in CRF mRNA expression in the PVN in response to ARS (61). This suggests that the anxiolytic and stress-attenuating effects of OXT are mediated by its inhibitory effect on CRF mRNA expression. Our recent study also showed that intracerebroventricular injection of OXT significantly attenuated the ARS-induced increase of CRF mRNA expression at the PVN (71). We also showed that delayed GE and accelerated CT induced by ARS were significantly improved by intracerebroventricular injection of OXT in mice (6) and rats (68, 71). In contrast to ARS, repeated experiences with the same stressor [chronic homotypic stress (CHS)] produce habituation or diminution of behavioral responses. We showed that the GI dysmotility observed in ARS was restored following CHS in rodents (6, 35, 71, 72). Although there is abundant evidence for a major role of OXT as an antistressor, the mechanisms through which GI dysmotility is restored following CHS remain unclear. We recently showed that OXT mRNA expression is upregulated, while CRF mRNA expression is downregulated, in the PVN following CHS in rats (68, 71). We also showed that central administration of OXT antagonists attenuated restored GI motility in mice (6) and rats (68, 71) following CHS. Furthermore, we demonstrated that CHS failed to restore delayed GE and accelerated CT in OXT knockout (KO) mice (3, 4). These findings suggest that central OXT is involved in mediating restored GI motility following CHS.

Exposure to stress in early life can induce an increased vulnerability to mood disorders later in life (52). Child maltreatment is a negative early life experience, associated with robust alterations in social behaviors, and acts as a risk factor for the development of excessive aggression and violence (19). Early life stress events have also been implicated as a risk factor for IBS (49). Exposure to early life stress induces visceral hyperalgesia and reduces somatic analgesia in re-
sponse to acute water avoidance stress in rats, mimicking the cardinal features of IBS (15).

In rodents, postnatal maternal separation (MS) of newborns is a well-established model of early life stress that results in permanent changes in the central nervous system (CNS) (15, 33). MS has been shown to increase HPA axis responsiveness to stressors by increasing CRF mRNA expression at the PVN (1). It has been shown that neonatal stress decreases OXT receptor binding in the CNS (33) and reduces the number of OXT neurons within the PVN (55). In addition to the CNS, MS-induced alterations in CRF receptors have been shown in the GI tract (44).

MS rats showed an enhanced fecal pellet output following acute water avoidance stress (15), suggesting increased colonic motility. However, the effect of early life stress on GI motility has not been fully studied. The present study was designed to investigate whether MS alters upper and lower GI motility in response to ARS and CHS.

OXT also plays an important role in the ability to form social attachments (8, 21, 70). Intranasal administration of OXT improves a person’s ability to infer the mental state of others (20) and communicate positively (18). Increased urinary OXT levels have been shown in children after physical contact with their mothers. In contrast, children who have been neglected do not exhibit an increase of urinary OXT (22). We recently showed that social interaction increases OXT mRNA expression and attenuates CRF mRNA expression at the PVN in adult rats (5).

We hypothesized that lack of physical and emotional contact between young pups and their mothers attenuates gene expression of OXT, which, in turn, impairs the adaptation process of GI motility to CHS. We assessed GE and CT under nonstressed (NS), ARS, and CHS conditions in male MS and nonhandled control rats. The changes in CRF and OXT gene expressions in the PVN were assessed in control and MS rats. We also studied the immunohistochemistry of OXT and CRF at the parvocellular and magnocellular subdivisions of the PVN following NS, ARS, and CHS.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (250–300 g body wt) were housed under conditions of controlled temperature (22–24°C) and illumination (12:12-h light-dark cycle, with lights on at 6 AM) for ≥7 days before the experiment. Rats were allowed ad libitum access to food and water. Protocols describing the use of rats were approved by the Institutional Animal Care and Use Committee of Zablocki Veterans Affairs Medical Center and carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and reduce the number of animals in experiments.

MS procedure. Pregnant Sprague-Dawley rats (n = 12) were supplied from the vendor 7–10 days before delivery. After delivery, the newborn pups (each litter contained 10–11 pups) underwent MS for 180 min from postnatal day (PND) 2 to PND 14, as previously described (47). The dams were transferred to new cages, and the pups were kept on heated pads in their home cages from 9 to 12 AM. The control pups were not handled and were kept with their dams. After they were weaned, the pups (each litter contained 6–7 males and 4–5 females) were sexed, and female pups were discarded on PND 21. Male control and MS pups were kept with their littersmates (2–6 rats/cage) until they were 8 wk old (250–300 g body wt). All the litters were from the first generation. Different subsets of rats were used for measurement of each behavioral, biochemical, and physiological parameter. Animals from different litters were distributed evenly through each experiment.

Elevated-plus-maze test. Animals were placed in the center of the maze facing an open arm, and all behaviors were recorded for 5 min with a video camera mounted above the maze. Anxiety-like behavior, locomotor activity, and risk assessment behavior were evaluated with the elevated-plus-maze test, as previously described (39). Anxiety-like behaviors were manifested with decreased exploration of open arms, while the total number of arm entries was recorded as an index of locomotor activity. The duration of scans, extension of the head over the edge of the open arms, and rearing while standing on the hindlimbs were recorded to evaluate risk-assessing behaviors.

ARS and CHS loading. Rats were placed on a wooden plate with their trunks wrapped in a confining harness for 90 min, as previously reported (72). The animals were able to move their limbs and head but not their trunks. This restraint stress has been used as a physical and psychogenic stress model in rodents (31, 60). For CHS, the rats received the same restraint stress for 5 consecutive days (72).

Solid GE study. For the evaluation of solid GE in response to ARS, rats were fasted for 24 h. Preweighed pellets (1.6 g) were given, as previously reported (72). Immediately after the feeding was completed, the rats were subjected to ARS for 90 min. The rats that did not consume 1.6 g of food within 10 min were excluded from the study. After the ARS loading, the rats were killed by an overdose of pentobarbital sodium (200 mg/kg ip). The stomach was surgically removed, and gastric contents were recovered, dried, and weighed. Solid GE was calculated as previously described (41, 72). For the evaluation of GE in response to chronic stress, a similar emptying study was performed following restraint stress loading on day 5 of CHS.

CT study. Rats were anesthetized by pentobarbital sodium (45 mg/kg ip). An in-dwelling Silastic cannula was inserted into the cecum and positioned to enter the proximal colon. The proximal portion of the tube was brought through the left abdominal wall and tunneled beneath the skin to the posterior neck and fixed to the skin. For postoperative pain relief, buprenorphine was administered (0.05 mg/kg sc) every 12 h for 2 days. At 1 wk after the surgery, a nonabsorbable radioactive marker (0.5 μCi, Na251CrO4 in 0.2 ml of saline) was introduced into the proximal colon, and rats were restrained for 90 min, as previously described (40).

After stress loading was completed, the rats were euthanized with an injection of pentobarbital sodium (200 mg/kg ip). The entire colon was surgically removed and divided into 10 equal segments. Each segment was placed in a vial, and radioactivity was counted by a gamma counter for 1 min. The geometric center (GC) of the distribution of 51Cr within the colon is the center of gravity for the distribution of radiochromium. GC was calculated as previously reported (40).

Quantitative RT-PCR of CRF and OXT. The rat brain tissue micropunching technique was used to acquire PVN from specific regions (1.8 mm caudal to bregma, 0.4 mm lateral to midline, 7.6 mm ventral to the brain surface), as previously reported (71). Total RNA was extracted from brain tissues using TRIzol (Invitrogen, Carlsbad, CA). Trace DNA contamination was removed by DNase (Promega, Madison, WI) digestion. cDNA was synthesized from 3 μg of total RNA using SuperScript III reverse transcriptase (Invitrogen). For amplification of rat CRF, OXT, and β-actin, the primers were designed according to previous reports (24, 67, 71) (Table 1). Quantitative PCR was performed using SYBR Premix Ex Taq (TakaraBio, Madison, WI). Amplification reactions were performed using a light cycler (model 480, Roche Diagnostics). Initial template denaturation was performed for 30 s at 95°C. The cycle profiles were programmed as follows: 5 s at 95°C (denaturation), 20 s at 60°C (annealing), and 15 s at 72°C (extension). Forty-five cycles of profile were run, and the final cooling step was continued for 30 s at 40°C. Quantitative measurement of each mRNA was achieved by establishment of a
linear amplification curve from serial dilutions of each plasmid containing the amplicon sequence. The relative amount of each mRNA was normalized by the amount of β-actin mRNA, as previously reported (71).

Immunohistochemistry of CRF and OXT. At 90 min after the restraint stress loading, rats were deeply anesthetized by pentobarbital sodium (200 mg/kg ip) and perfused intracardially with 0.15 M phosphate buffer (PB) followed by an ice-cold fixative of 4% paraformaldehyde in 0.1 M PB. The brain was removed and immersed for 24 h in 4% paraformaldehyde in 0.1 M PB. The brain was then placed in 30% sucrose and PBS for ≥48 h for cryoprotection. The tissue was cut into 40-μm-thick coronal sections on a cryostat, and sections containing PVN were sampled according to the brain atlas by Paxinos and Watson (46). Tissues were washed with PBS (3 times for 5 min each). Sections were blocked for 1 h in 0.15 M PBS containing 4% normal goat serum and 0.2% Triton X-100 and incubated with coverslips were mounted with Entellan.

Table 1. Sense and antisense primers used in RT-PCR

<table>
<thead>
<tr>
<th>Sense</th>
<th>Antisense</th>
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<tr>
<td>CRF</td>
<td>5’-CCAGGGCCAGCCAGTCTAGCT-3’</td>
</tr>
<tr>
<td>OXT</td>
<td>5’-GAGAGGGAGCCATGCGGG-3’</td>
</tr>
<tr>
<td>β-Actin</td>
<td>5’-TGGGACAGAGACCCCTTGTCAATA-3’</td>
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Sense and antisense primers for corticotropin-releasing factor (CRF) (24, 71), oxytocin (OXT) (67, 71), and β-actin (71) have been reported previously.

Effect of MS on body weight. Body weight was significantly lower in juvenile (2- and 3-wk-old) and adult (8-wk-old) MS than control rats (n = 10–14, P < 0.01; Table 2).

Table 2. Effect of MS on body weight in juvenile and adult rats

<table>
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<tr>
<th>Group</th>
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<th>3 week old</th>
<th>8 week old</th>
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<tr>
<td>Control</td>
<td>10</td>
<td>35.9 ± 0.4</td>
<td>61.2 ± 1.5</td>
<td>327.5 ± 5.3</td>
</tr>
<tr>
<td>MS</td>
<td>14</td>
<td>30.9 ± 0.9*</td>
<td>51.9 ± 1.4*</td>
<td>294.6 ± 5.1*</td>
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Values are means ± SE. Maternal separation (MS) significantly reduced body weight in 2- and 3-wk-old (juvenile) and 8-wk-old (adult) rats compared with controls: *P < 0.01 vs. control.

RESULTS

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Effect of MS on anxiety-like behavior and locomotor activity. MS rats showed a significant decrease (6.8 ± 1.0% of 5 min, n = 18, P < 0.01) in time spent in open arms compared with control rats (29.7 ± 2.9%, n = 10) (Fig 1A). MS rats also showed a significantly reduced number of total arm entries (4.4 ± 0.5, n = 18, P < 0.01) compared with controls (13.6 ±

Fig. 1. Effect of maternal separation (MS) on anxiety-like behavior (A), locomotor activity (B), and risk assessment behavior (C) in adult rats. MS significantly decreased the percentage of time spent in open arms and total number of arm entries, whereas it increased the total time of risk-assessing behaviors compared with controls. Values are means ± SE (n = 10 control and 18 MS rats). **P < 0.01 vs. control.
Effect of MS on solid gastric emptying (GE, A) and colonic transit (CT, B) under nonstressed (NS), acute restraint stress (ARS), and chronic homotypic stress (CHS) conditions. Compared with NS, ARS significantly delayed GE in control and MS rats (A). After CHS, delayed GE observed under ARS completely recovered to basal levels in control, but not MS, rats. ARS significantly accelerated CT in control and MS rats (B). Accelerated CT was completely restored in control rats following CHS. In contrast, CT was still accelerated in MS rats following CHS. CT is shown as geometric center (GC). Values are means ± SE (n = 5–7). *P < 0.05 vs. each group. **P < 0.01 vs. NS of each group.

Effect of MS on GE in response to ARS and CHS. In nonstressed (i.e., NS) conditions, GE was 53.0 ± 0.6% (n = 6) and 58.4 ± 1.5% (n = 7) in control and MS rats, respectively. ARS significantly delayed GE in control (30.3 ± 3.3%, n = 6, P < 0.01) and MS (36.8 ± 3.3%, n = 6, P < 0.01) rats. The delayed GE was completely restored to 53.1 ± 3.5% (n = 7) in control rats following CHS. In contrast, GE was still delayed (31.5 ± 2.8%, n = 6, P < 0.01) in MS rats following CHS (Fig. 2A).

Effect of MS on CT in response to ARS and CHS. In NS conditions, GC was 5.6 ± 0.5 (n = 6) and 5.9 ± 0.2 (n = 6) in control and MS rats, respectively. ARS significantly accelerated CT in control (GC = 8.6 ± 0.1, n = 6, P < 0.01) and MS (GC = 7.9 ± 0.3, n = 6, P < 0.01) rats. The accelerated CT was completely restored in control rats (GC = 6.1 ± 0.4, n = 6) following CHS. In contrast, CT was still accelerated (GC = 9.1 ± 0.5, n = 6, P < 0.01) in MS rats following CHS (Fig. 2B).

Effects of MS on hypothalamic CRF mRNA and OXT mRNA expression in response to ARS and CHS. In NS conditions, there was no significant difference in CRF mRNA expression between control and MS rats. In response to ARS, CRF mRNA expression in the PVN was significantly increased in control and MS rats (n = 5, P < 0.05). After CHS, the increased CRF mRNA expression was reduced to control levels in control, but not MS, rats (Fig. 3A).

OXT mRNA expression in the PVN was significantly increased following ARS in control and MS rats. In contrast, MS rats failed to increase OXT mRNA expression in response to CHS (Fig. 3B).

Correlation between GE and CRF and between GE and OXT mRNA expression in response to CHS. After CHS, there was a significant negative correlation between GE and CRF mRNA expression in the PVN in control and MS rats (n = 5, P < 0.01; Fig. 4A). In contrast, a significant positive correlation was observed between GE and OXT mRNA expression (n = 5, P < 0.01; Fig. 4, A and B) in control and MS rats.

Correlation between CT and CRF and between CT and OXT mRNA expression in response to CHS. After CHS, there was a significant positive correlation between CT and CRF mRNA expression in the PVN in control and MS rats (n = 5, P < 0.01; Fig. 4A). In contrast, a significant negative correlation was observed between CT and OXT mRNA expression (n = 5, P < 0.01) in control and MS rats (Fig. 4D).

Immunohistochemistry of CRF at the PVN in MS rats in response to ARS and CHS. The number of CRF-immunoreactive cells at the paraventricular [posterior magnocellular (PM)] subdivision of the PVN was reduced following CHS in control rats (Fig. 5C). In contrast, MS rats still showed an increased number of CRF-immunoreactive cells (Fig. 5C).
Statistical analysis showed no difference in the number of CRF-immunoreactive cells in the parvocellular subdivision of the PVN of control (77.5 ± 13.5, n = 5–7) and MS (74.2 ± 8.6, n = 5–7) rats under NS conditions. In response to ARS, the number of CRF-immunoreactive cells was significantly increased in control (158.5 ± 30.2, n = 5–7, P < 0.01; A) and MS (145.5 ± 8.6, n = 5–7, P < 0.05, P < 0.01) rats. However, in response to CHS, the increased number of CRF-immunoreactive cells in control rats (101.5 ± 7.3, n = 5–7) was significantly reduced. In contrast, MS rats still showed an increased number of CRF-immunoreactive cells (161.7 ± 20.2, n = 5–7, P < 0.05; Fig. 6).

**Fig. 4. Correlation between GE and CRF mRNA (A), GE and OXT mRNA (B), CT and CRF mRNA (C), and CT and OXT mRNA (D) expression following CHS.** Note significant negative correlation between GE and CRF mRNA expression (n = 5, P < 0.05; A) and between CT and OXT mRNA expression (n = 6, P < 0.05; D) and significant positive correlation between GE and OXT mRNA expression (n = 5, P < 0.01; B) and between CT and CRF mRNA expression (n = 5, P < 0.05; C). CT is shown as geometric center (GC).

**Fig. 5. Immunohistochemistry of CRF in control and MS rats following NS (A), ARS (B), and CHS (C) at the PVN. 3V, 3rd ventricle. Scale bars, 250 μm.**

**Immunohistochemistry of OXT at the PVN in MS rats in response to ARS and CHS.** The number of OXT-immunoreactive cells at the anterior magnocellular (AM) and PM subdivisions of the PVN was remarkably increased following CHS (Fig. 7C) in control rats. MS rats did not show any significant increase in the OXT-immunoreactive cells (Fig. 7C).
Statistically, there were no significant changes in OXT-immunoreactive cells in the parvocellular region of the PVN following NS, ARS, and CHS in the control and MS rats (Fig. 8A).

There was no significant difference in the number of OXT-immunoreactive cells at the AM subdivision of the PVN between control (18.8 ± 8.9, n = 5–7) and MS rats (21.2 ± 9.1, n = 5–7) in NS conditions (Fig. 8B). In control (37.2 ± 10.0, n = 5–7, P < 0.05) and MS (38.7 ± 5.2, n = 5–7) rats, the number of OXT-immunoreactive cells at the AM subdivision of the PVN was slightly increased following ARS compared with NS. After CHS, the number of OXT-immunoreactive cells at the AM subdivision was remarkably increased in the control rats (52.1 ± 6.8, n = 5–7, P < 0.05). In contrast, MS rats still showed smaller number of OXT-immunoreactive cells (32.2 ± 2.9, n = 5–7, P < 0.05; Fig. 8B).

At the PM subdivision of the PVN, ARS increased the number of OXT-immunoreactive cells in control (75.2 ± 16, n = 5–7, P < 0.05) and MS (71.0 ± 9, n = 5–7) rats compared with NS. However, after CHS, the number of OXT-immunoreactive cells at the PM subdivision showed a pronounced increase in control rats (117.6 ± 15, n = 5–7). In contrast, MS failed to increase the number of OXT-immunoreactive cells (67.3 ± 12, n = 5–7, P < 0.05; Fig. 8C).

**DISCUSSION**

Stressful social experiences in early life and social isolation have enduring effects on the development of the brain and behavior (45). MS is widely used as a model of early life stress, with long-lasting alterations in anxiety-like behaviors (47, 52, 59). MS rats show anxiety-like behaviors, which are characterized by spending less time in the open arms (28), increased explorative and risk assessment behaviors (50), and decreased locomotor activity (32) in the elevated-plus-maze test.

In line with these previous findings, our MS rats spent less time in the open arms and had less arm entries than controls. Moreover, MS rats showed more exploratory risk assessment behavior than control rats.

In response to stress, CRF is secreted from the hypothalamus and activates the HPA axis, resulting in secretion of corticosterone from the adrenal cortex to guard against stress disorders (23). ARS delays GE (41, 54), while it accelerates CT, in
ance stress-induced stimulation of colonic motility is attenuated by central, but not peripheral, administration of OXT (36). These data suggest that the central OXT receptors play a dominant role in regulating acute stress-induced GI dysmotility.

Plotsky and Meaney (47) reported increased CRF mRNA expression at the PVN and increased CRF-like immunoreactivity in the cerebrospinal fluid have been shown in MS rats under NS conditions. In contrast, Desbonnet et al. (17) others showed no difference in plasma corticosterone and hypothalamic CRF levels between MS and control male rats under acute stress conditions. We did not observe significant differences in CRF mRNA expression at the PVN between MS and control rats under NS conditions. Similarly, OXT mRNA expression was not significantly different between control and MS rats under NS conditions. There were also no significant differences in GE and CT under NS conditions.

In response to ARS, GE was delayed and CT was accelerated to a similar degree in control and MS rats. In contrast, delayed GE and accelerated CT in ARS were completely restored following CHS in control rats, while delayed GE and accelerated CT were still observed in MS rats, suggesting maladaptation to CHS.

ARS remarkably increased CRF and OXT mRNA expression in both groups. In response to CHS, CRF mRNA expression was returned to the basal levels in control rats, while CRF mRNA expression was still elevated in MS rats. On the other hand, OXT mRNA expression was much more pronounced in control rats in response to CHS. MS rats failed to increase OXT mRNA expression following CHS.

MS increases the HPA axis responsiveness to stressors by increasing CRF mRNA expression in the PVN (1). MS up-regulates hypothalamic CRF1 receptor expression in rats, suggesting that CRF-induced activation of the CRF1 receptor may contribute to the hyperactive HPA axis in MS rats (43). Neonatal stress decreases OXT receptor binding in the CNS (33) and reduces the number of OXT neurons within the PVN (55). However, it remains unknown whether altered gene expression of CRF and OXT is involved in regulating the stress response of the GI tract.

It has been shown that the stress-attenuating effect of OXT is mediated by its inhibitory effect on CRF mRNA expression (61). We showed that intracerebroventricular injection of OXT inhibits CRF mRNA expression at the PVN (71). We also showed that inhibitory effects of OXT on CRF expression are mediated via GABA_A receptors at the PVN in rats (10). Our previous studies suggest that the adaptation mechanism of GI motility in response to CHS involves upregulation of OXT expression and downregulation of CRF expression in rodents (6, 68, 71). We showed a significant negative correlation between CRF mRNA and OXT mRNA expression following AS and CHS in rats (71) and mice (6). Similarly, our current study shows a significant negative correlation between OXT and CRF mRNA expression in the PVN following CHS in control and MS rats. A positive correlation between OXT mRNA expression and GE and a negative correlation between CRF mRNA expression and GE were observed following CHS in control and MS rats. Thus the maladaptation of GI motility in MS rats can be explained by upregulated CRF expression and downregulated OXT expression.

At the PVN level, OXT is produced in the magnocellular and parvocellular neurons, while CRF is produced in the rodents (38, 40). We previously showed that delayed GE induced by ARS is mediated via central CRF type 2 receptors and sympathetic pathways (41). In contrast, accelerated CT induced by ARS is mediated via central CRF type 1 (CRF1) receptors and parasympathetic pathways in rats (40). Although the motor responses to ARS differ between the upper and the lower GI tract, both responses are mediated via the same neuropeptide of the CNS, i.e., CRF.

We recently demonstrated that intracerebroventricular administration of OXT, which had little effect on GE under normal conditions, significantly improved delayed GE induced by ARS in mice (6) and rats (71). It is, therefore, suggested that central OXT receptors may play a predominant role in regulating stress-induced GI dysmotility, but not in NS conditions. OXT receptors are also expressed on the smooth muscle cells (48), enteric neurons, and intestinal epithelium (58) of the GI tract in rats. We showed that intracerebroventricular, but not intraperitoneal, injection of OXT improved gastric dysmotility induced by ARS (11). Others showed that acute water avoidance stress-induced stimulation of colonic motility is attenuated by central, but not peripheral, administration of OXT (36). These data suggest that the central OXT receptors play a dominant role in regulating acute stress-induced GI dysmotility.

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ARS remarkably increased CRF and OXT mRNA expression in both groups. In response to CHS, CRF mRNA expression was returned to the basal levels in control rats, while CRF mRNA expression was still elevated in MS rats. On the other hand, OXT mRNA expression was much more pronounced in control rats in response to CHS. MS rats failed to increase OXT mRNA expression following CHS.

MS increases the HPA axis responsiveness to stressors by increasing CRF mRNA expression in the PVN (1). MS up-regulates hypothalamic CRF1 receptor expression in rats, suggesting that CRF-induced activation of the CRF1 receptor may contribute to the hyperactive HPA axis in MS rats (43). Neonatal stress decreases OXT receptor binding in the CNS (33) and reduces the number of OXT neurons within the PVN (55). However, it remains unknown whether altered gene expression of CRF and OXT is involved in regulating the stress response of the GI tract.

It has been shown that the stress-attenuating effect of OXT is mediated by its inhibitory effect on CRF mRNA expression (61). We showed that intracerebroventricular injection of OXT inhibits CRF mRNA expression at the PVN (71). We also showed that inhibitory effects of OXT on CRF expression are mediated via GABA_A receptors at the PVN in rats (10). Our previous studies suggest that the adaptation mechanism of GI motility in response to CHS involves upregulation of OXT expression and downregulation of CRF expression in rodents (6, 68, 71). We showed a significant negative correlation between CRF mRNA and OXT mRNA expression following AS and CHS in rats (71) and mice (6). Similarly, our current study shows a significant negative correlation between OXT and CRF mRNA expression in the PVN following CHS in control and MS rats. A positive correlation between OXT mRNA expression and GE and a negative correlation between CRF mRNA expression and GE were observed following CHS in control and MS rats. Thus the maladaptation of GI motility in MS rats can be explained by upregulated CRF expression and downregulated OXT expression.

At the PVN level, OXT is produced in the magnocellular and parvocellular neurons, while CRF is produced in the
parvocellular neurons (53). Synaptic associations between CRF and magnocellular OXT neurons of the PVN have also been demonstrated (27). Forced swimming stress increases OXT mRNA expression in the magnocellular, but not parvo-
cellular, neurons of the PVN in rats (64). Restraint stress induces c-fos expression in OXTergic magnocellular neurons in the supraoptic nucleus and PVN in rats (37).

We previously showed that the number of OXT-immunoreactive cells at the PM and medial parvocellular subdivisions of the PVN was remarkably increased following CHS compared with ARS (71). Our recent in situ hybridization study also demonstrated that the anatomic changes of OXT synthesis (translation) were well correlated with its gene expression (transcription) (68). In the present study, we demonstrated that MS rats failed to show a significant increase of OXT-immunoreactive cells following CHS. The number of CRF-immunoreactive cells at the parvocellular subdivisions of the PVN was increased following ARS in control and MS rats. In contrast, the increased number of CRF-immunoreactive cells was reduced following CHS in control rats, while MS rats still showed an increased number of CRF-immunoreactive cells.

As shown in Fig. 3, mRNA expression of CRF and OXT was quickly increased in response to 90 min of exposure to ARS. The number of CRF-immunopositive cells, but not the number of OXT-immunopositive cells, was significantly increased in response to ARS. The quick increase of CRF-immunopositive cells at the PVN was also reported in response to 60 min of water avoidance stress in rats (36). Others showed that urocortin 1-immunoreactive cells in the midbrain, but not CRF-
immunopositive cells in the PVN, are increased within 60–120 min after acute pain stress in rats (51). Thus the process of transcription and translation may depend on each neuropeptide and type of stress.

In 3-wk-old MS rats, body weight was reduced but food intake was not affected, and a rebound hyperphagia in 6-, 9-, and 12-wk-old rats compensated for this weight loss (29). Ghrelin stimulates feeding behavior in rats (65, 66), and its secretion is upregulated under negative energy balance (13, 56). Plasma acylated ghrelin and gastric ghrelin mRNA expressions were lower in MS adolescent rats than nonhandled controls (16). In our current study, a significant reduction of body weight was observed in 2-, 3-, and 8-wk-old MS rats. It is not clear whether the reduction of body weight is attributed to reduced food intake or decreased ghrelin secretion in MS rats.

OXT plays a key role in regulating positive social behaviors, including sexual behavior, mother-infant and adult-adult pair-

bond formation, and social memory/recognition. OXT KO mice are more aggressive than wild-type mice. OXT KO mice fail to recognize familiar conspecifics after repeated social encounters (63).

Autism is a developmental disorder characterized by speech and communication abnormalities, social functioning impair-
ments, and repetitive behaviors and restricted interests (7). There was a decrease in plasma OXT in the autistic sample compared with control subjects. Deficits in OXT peptide pro-
cessing in children with autism may be important in the development of this syndrome (25). Compared with placebo, OXT administration with nasal spray improves emotion rec-
ognition in young people diagnosed with autism spectrum disorders (26). Although early life stress events have been implicated as a risk factor for autism, it remains to be seen whether OXT expression and GI motility in response to stress are altered in autism.

Two-thirds of individuals with IBS are female, and it has been suggested that sex difference mediates stress-induced GI dysmotility (12). Previous studies reported higher glucocorti-
coid levels in female than male rats after HPA axis activation (69). We recently compared the effects of ARS and CHS on CT between male and female rats. ARS accelerated CT, which was completely restored to normal levels following CHS to the same degree in male and female rats (68). As no sex differ-
ences were observed in CT, we used male rats in this study. However, further study is needed to elucidate the sex differ-
ence in mediating GI dysmotility in MS rats.

Exposure to stressful events in early life stages may increase vulnerability of the GI tract in later stages. Our current study is the first report that revealed MS-induced impaired adaptation response of GE and CT following CHS. Epidemiological studies suggest considerable overlap between FD and IBS. About half of the FD patients fulfill the criteria for IBS (14). We propose that the restoration of gastric and colonic dysmo-
tility in CHS occurs through the mechanisms of upregulation of OXT and attenuation of CRF expression of the hypothalamus. Our study may contribute to a better understanding of the mechanism and treatment of functional GI disorders, both FD and IBS, associated with early life stress.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
M.B., R.B., D.C., and S.Y. performed the experiments; M.B., R.B., and T.T. prepared the figures; R.B., K.L., and T.T. drafted the manuscript; M.B., R.B., and T.T. analyzed the data; M.B., R.B., and T.T. interpreted the results of the experiments; R.B., K.L., and T.T. are responsible for conception and design of the research; R.B. and T.T. interpreted the results of the experiments; R.B. and T.T. drafted the manuscript; T.T. approved the final version of the manuscript.

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