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Repeated psychological stress-induced alterations of visceral sensitivity and colonic motor functions in mice: Influence of surgery and postoperative single housing on visceromotor responses

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Abstract

Visceral pain modulation by chronic stress in mice has been little studied. Electromyography (EMG) recording of abdominal muscle contractions, as a proxy to the visceromotor response (VMR), requires electrode implantation and post-surgical single housing (SH) which could affect the VMR to stress. To test this hypothesis, male mice had electrode implantation surgery (S) plus SH, or no surgery and were group housed (NS-GH) or single housed (NS-SH) and exposed to either water avoidance stress (WAS, 1 h/day) or left undisturbed in their home cages for 10 days. The VMR to phasic ascending colorectal distension (CRD) was assessed before (basal) and 24 h after 10 days of WAS or no stress using a surgery-free method of intraluminal colonic pressure (ICP) recording (solid-state manometry). WAS heightened significantly the VMR to CRD at 30, 45, and 60 mmHg in S-SH vs. NS-GH, but not compared to NS-SH conscious mice. Compared to basal CRD, WAS increased VMR at 60 mmHg in the S-SH group and decreased it at 30–60 mmHg in NS-GH mice, while having no effect in NS-SH mice. The average defecation during the hour of repeated WAS over 10 days was 1.9 and 2.4 fold greater in S-SH vs. NS-GH and NS-SH mice, respectively. These data indicate that the combination of S-SH required for VMR monitoring with EMG is an important component of repeated WAS-induced post-stress visceral hypersensitivity and defecation in mice.

Keywords

Chronic water avoidance stress; colonic motor function; defecation; irritable bowel syndrome; social isolation; visceral pain

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Introduction

Chronic stress plays a major role in the onset, maintenance, and exacerbation of irritable bowel syndrome (IBS) symptoms (abdominal pain and alterations in bowel habits) (Hertig et al. 2007). Although preclinical studies have helped identify some potential pathways at play in the influence of stress on visceral hypersensitivity, most investigations have been performed in the context of acute stress (Taché et al. 2004; Buéno 2008; Kakol-Palm et al. 2008; Storr et al. 2008). In addition, despite the availability of genetically modified mice with relevance to the pathophysiology of IBS (Million et al. 2006a; Trimble et al. 2007), murine models to investigate influence of chronic stress on visceral hyperalgesia and colonic motor function have been little explored (Fioramonti and Gebhart 2007). In rats, repeated water avoidance stress (WAS), a psychological stressor, alters the physiology of the gastrointestinal tract (Söderholm et al. 2002; Yang et al. 2006) and leads to a concomitant sustained visceral hypersensitivity monitored by electromyography (EMG) recordings (Bradesi et al. 2005). Similarly, chronic WAS alters the colonic barrier epithelial function of Balb/c and C57Bl/6 mice (Cameron and Perdue 2005; Melgar et al. 2008). However, a recent report indicates that chronic WAS does not affect the visceral sensitivity to colorectal distension (CRD) when monitored with manometry in conscious female mice (Larsson et al. 2009).

While extensively used in rats (Ness and Gebhart 1988; Ait-Belgnaoui et al. 2005), the assessment of the visceromotor response (VMR) to isobaric CRD by EMG recording of the electrical activity changes in the abdominal musculature has only been recently developed for mice (Kamp et al. 2003; Larsson et al. 2003; Christianson and Gebhart 2007; Martinez and Melgar 2008). This method, however, is technically challenging in mice (Christianson and Gebhart 2007) due to their small size and potential complications, particularly in genetically modified mice that are more sensitive to surgical interventions. In addition, it requires post-surgical single housing (SH) to prevent damage to the EMG electrodes by cage mates. These experimental and environmental conditions may introduce additional confounding factors in the parameters recorded, particularly under conditions of chronic stress investigations. There is evidence that SH influences the reactivity of mice to an additional mild stressor (Palanza et al. 2001; Bartolomucci et al. 2003). Moreover, EMG surgery involves skin and/or muscle incision depending on the technique used (subcutaneous abdominal electrodes or intraperitoneal cannula) and chronic implantation of a foreign body. Even though no data are available in the literature in relation to chronic EMG electrodes in the abdominal wall, such intervention could induce a host–tissue response with local micro-inflammation (neutrophils, lymphocytes, and macrophages) as it has been shown for other types of implants in the skin and peritoneum (Marois et al. 1993; Klueh and Kreutzer 2005). The endogenous substances (e.g. inflammatory mediators, growth factors, and endothelins) hence released could increase the excitability of nociceptors (Banik et al. 2005; Khodorova et al. 2009; Wu et al. 2009) and affect the visceral sensitivity. Nevertheless, there is a paucity of alternative methods to measure VMR to CRD in conscious mice. Initial studies to monitor visceral pain in mice were based on visual inspection of the abdominal activity (Martinez et al. 1999) or behavioral changes as surrogate marker for visceral pain (Laird et al. 2001) in response to intracolonic or intraperitoneal injections of irritants. However, behavioral monitoring can be subjective and is difficult to master especially in small animals like mice that present a vast repertoire of behaviors. Mice pain behavior can also be affected by the presence of the experimenter in the room, which makes the monitoring more difficult (Langford et al. 2006). In addition, in these previous mice studies, the visceral pain response induced by chemical irritants and nociceptive CRD differs (Martinez et al. 1999; Laird et al. 2001). Lastly, while the method is well established and used in rats (Al-Chaer et al. 2000), the assessment of visually detectable abdominal contractions in response to CRD cannot be done in mice (Martinez et al. 1999; Larsson et al. 2003). To date only a single research

group, from a research and development pharmaceutical company, reports the use of a non-invasive manometric technique in conscious restrained mice using custom-made equipment (Arvidsson et al. 2006; Martinez and Melgar 2008; Brusberg et al. 2009).

In the present study, we first validated an alternative non-invasive method to study visceral sensitivity to CRD in conscious mice, using a commercially available miniaturized pressure catheter to record intraluminal colonic pressure (ICP). This was achieved by monitoring VMR to graded phasic CRD by simultaneously recording ICP and EMG signals in mice chronically implanted with electrodes. As colonic pressure could be altered following abdominal contractions and/or contractions of the colonic wall proper, we assessed the effects of atropine, a muscarinic blocker known to inhibit colonic motility in mice (Gourcerol et al. 2009), on the VMR to CRD assessed by ICP in naïve mice. In addition, to examine the reliability and validity of the methods to detect changes in visceral pain, we used buprenorphine, a partial agonist for mu-opioid receptors (Kamei et al. 1995; Christoph et al. 2005) with clear analgesic properties and assessed the VMR to CRD. Then, using the validated ICP method, we tested the hypothesis that a repeated psychological stress may have differential influence on the VMR to CRD and colonic propulsive motor function, as monitored by defecation in conscious mice that underwent EMG surgery and thereafter SH vs. mice that underwent no surgery and were single or group housed.

Materials and methods

Animals

Adult male C57Bl/6 mice (25–30 g) (Harlan Laboratory, San Diego, CA, USA) housed in groups (2–4/cage), except otherwise indicated, were maintained under standard housing conditions of illumination (12:12 h light/dark cycle, lights on at 06:00 h) with water and chow (Purina®) *ad libitum*, and acclimated to the animal facility for 1 week before the start of the experiments. NIH guidelines were followed and all experiments were undertaken under the auspices of an OLAW Assurance of Compliance (A3002-01) and performed according to approved protocols by the IACUC Committee of the VA Greater Los Angeles Healthcare System (VAGLAHS) (99127-07).

Psychological stress

The procedure for WAS was essentially conducted as described previously in mice (Cameron and Perdue 2005; Melgar et al. 2008). Exposure to WAS was performed between 09:00 h and 12:00 h for 1 h per day for 10 consecutive days with all groups tested run in parallel to control for experimental variations. WAS conditions consisted of placing mice individually on a rectangular platform (3 cm length × 3 cm width × 6 cm height, AMAC box M series #510C, AMAC Plastic, Petaluma, CA, USA) affixed in the center to the bottom of a container (30.8 cm length × 30.8 cm width × 20.6 cm height, Medium FunBox #1636, Sterilite, Townsend, MA, USA) filled with room temperature tap water up to 1 cm below the top of the platform. Mouse body weight (BW) was monitored daily before each stress session and the percentage of daily individual weight change was calculated as follows: $(BW_{\text{day}x} - BW_{\text{day}0})/BW_{\text{day}0} \times 100$.

Fecal pellet output

The fecal pellet output (FPO) was determined as the cumulative number of fecal emissions for the 1-h WAS session each day of the 10 days of repeated WAS in the time course study. Additionally, mean 1-h output was calculated by averaging the total number of fecal emissions over the 10 days of WAS. Basal defecation of mice was determined in separate groups of mice not subjected to stress, while being kept for an hour in their home cage according to their test-housing configuration (group housed or single housed), after a quick

bedding change at the same time of the day during which stress experiments were performed. For group-housed mice, defecation was averaged by the number of mice in the cage.

Assessment of visceral pain during CRD

Mice were anesthetized with intraperitoneal (i.p.) injections of ketamine (80 mg/kg 0.1 ml, Ketaset, Fort Dodge Laboratories Inc., Fort Dodge, IA, USA) and xylazine (12 mg/kg 0.05 ml, Rompun, Mobay Corporation, Shawnee, KS, USA) and electrodes for EMG recordings were chronically implanted as previously described (Kamp et al. 2003; Cenac et al. 2007; Gecse et al. 2008). A group of three nichrome electrodes (40 AWG Nichrome 80 w/red Poly Enamel, Pelican Wire Company, Naples, FL, USA) was implanted into the external oblique abdominal muscle, then exteriorized in the back of the neck and protected by a plastic tube attached to the skin. Following surgery, mice were housed singly and allowed to recover for 5 days before the experiments.

A PE50 catheter was taped 2 cm below the pressure sensor of a miniaturized pressure transducer catheter (SPR-524 Mikro-Tip catheter; Millar Instruments, Houston, TX, USA). A custom-made balloon (1 cm width $\times$ 2 cm length) (Kamp et al. 2003; Arvidsson et al. 2006; Christianson and Gebhart 2007) prepared from a polyethylene plastic bag was tied over the catheter at 1 cm below the pressure sensor with silk 4.0. Ligature points were covered with parafilm to prevent any air leak (Figure 1(A)). At the start of each experiment, each “balloon-pressure sensor” was calibrated at known pressures of 0, 20, 40, and 60 mmHg using a barostat (Distender Series II, G&J Electronics Inc. Toronto, Canada), and voltage output was converted to pressure using CED digital analog converter (Micro 1401, Cambridge Electronic Design, Cambridge, UK) and Spike 2 software (CED, Ltd., Cambridge, UK).

On experimental day, mice were briefly anesthetized with isoflurane (3% in O₂) and the lubricated “balloon-pressure sensor” was introduced into the colorectum such that the distal end of the balloon was at 0.5 cm past the anus and secured to the tail with tape. Each mouse was placed in an adjustable mouse restrainer (3.3 cm diameter $\times$ 9 cm length, #51325, Stoelting Co, Wood Dale, IL, USA), covered with a light tissue blanket and left to rest for 30 min before the CRD procedure. Each balloon was connected to the barostat and the miniaturized pressure transducer to a preamplifier (model 600; Millar Instruments, Houston, TX, USA). The EMG electrodes were inserted into an electrode board connected to an electroencephalograph (Grass Instrument Co., Quincy, MA, USA) where the signal was amplified ($\times 10,000$). Both signals were acquired using the CED Micro1401/SPIKE2 program. The EMG signal was filtered (1000 Hz), digitized, and rectified (Million et al. 2006b). The CRD protocol consisted of a set of graded phasic distensions to constant pressures of 15, 30, 45, and 60 mmHg (three times each, 10 s duration, 4 min inter-stimulus interval). A similar CRD paradigm has been used previously to assess pain-related responses in mice (Kamp et al. 2003; Christianson and Gebhart 2007).

The data analysis of the traces was performed as follows. The phasic component of the ICP (pICP) was extracted from the ICP signal recorded by applying the “DC Remove” process in Spike 2 with a time constant of 1 s, to exclude the slower, tonic changes in ICP resulting from colonic smooth muscle activity, and by applying the “RMS amplitude” process with a time constant of 1 s to the resulting trace. Both EMG and ICP activities were recorded for 10 s before (non-distended ICP), during, and after termination of CRD. The VMR was defined as the increase in area under the curve (AUC) of pICP or EMG during CRD over the mean value of pre- and post-distension periods and was quantified using the “modulus” process in Spike 2. As each CRD pressure was repeated three times, the pre-post CRD and during CRD values were averaged for each pressure. To examine the pressure—response

relationship and adjust for inter-individual variations of the signal (Christianson and Gebhart 2007), ICP amplitudes were normalized for each mouse to the highest pressure (60 mmHg) in the first set of CRD. This value served as 100% response (control) in the baseline period of data collection before exposure to WAS or treatment and represented the baseline VMR. The VMR to the second CRD after WAS or treatment was expressed either as percentage from their normalized control values (percentage control) or mean change from the baseline response (Δ VMR in percentage control) at different distension pressures as validated in our previous studies (Larauche et al. 2009). It is known that the EMG signal can acquire electrical noise (Raez et al. 2006). Hence for data to be analyzed, the signal-to-noise ratio should be high: we excluded from the study mice equipped with EMG electrodes showing an EMG signal/noise ratio < 0.5 (Larauche et al. 2008). The signal/noise ratio was calculated as follows: $\text{signal/noise} = (\text{value during CRD} - \text{mean pre/post CRD value}) / \text{mean pre/post CRD value}$.

Validation of ICP as a measure of visceral pain in conscious mice

Simultaneous recording of EMG and ICP signals to graded CRD—Mice were chronically equipped with EMG electrodes and single housed thereafter for 5 days. Then, in mice briefly anesthetized by isoflurane inhalation, the lubricated “balloon-pressure sensor” was inserted into the colorectum and the animal was placed in a mouse restrainer. The balloon was connected to the barostat, the miniaturized pressure transducer to a preamplifier, and the EMG electrodes inserted into an electrode board connected to an electroencephalograph. The correlation between EMG and ICP signals as a function of pressure was assessed in response to ascending phasic CRD (15, 30, 45, and 60 mmHg, three times each, 10 s duration, 4 min inter-stimulus interval). For every mouse, the pressure threshold for both EMG and ICP responses was defined as the pressure of the distending pulse at which the response evoked exceeded the mean baseline activity plus three times the SD (Arvidsson et al. 2006).

Effects of buprenorphine and atropine on the VMR response to CRD monitored by the ICP method

In naïve mice, a first set of CRD was performed. After a 1-h rest period, an intraperitoneal (i.p. 0.1 ml) injection of either buprenorphine (0.3 mg/kg; Bedford Labs, Bedford, OH, USA, $n = 10$) or saline (i.p. $n = 9$) or a subcutaneous (s.c. 0.1 ml) injection of atropine sulfate (1 mg/kg; Sigma-Aldrich, St Louis, MO, USA, $n = 10$) was performed and 15 min later a second set of CRD was performed. The dose of atropine selected was based on previous studies showing that a similar regimen of administration inhibits colonic motility in conscious mice (Arvidsson et al. 2006; Gourcerol et al. 2009). The dose and route of buprenorphine were selected based on previous reports showing efficacy in models of acute pain (Kamei et al. 1995; Christoph et al. 2005).

Influence of surgery and SH on visceral responses to repeated WAS

For the study of visceral sensitivity, naïve mice were subjected to a first set of CRD and then divided in three subgroups; one group (surgery-single housed, S-SH, $n = 13$) was subjected to EMG surgery and housed singly, immediately after surgery, until the end of the experiments. A 5-day recovery period was allowed before beginning WAS. The second and third groups had no surgery and were maintained either group housed (NS-GH, $n = 18$) or single housed (NS-SH, $n = 10-13$). All groups were exposed to 1 h WAS each day for 10 consecutive days and daily BW and FPO were monitored during each stress session. On day 11, 24 h after the last exposure to WAS, a second set of CRD was performed on all groups of mice. Assessment of basal defecation was performed in separate groups of mice subjected

as previously to S-SH ($n = 5$), NS-GH ($n = 4$), or NS-SH ($n = 13$) except that they did not undergo visceral pain testing nor daily WAS.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). Linear regression analysis (R^2) and Pearson's correlation (R_p) were performed to determine the coefficient of correlation between pICP and EMG measurements when recorded simultaneously on the same mouse. The effect of buprenorphine, saline, atropine, or repeated WAS on ICP responses to the second set of CRD within one group of mice was analyzed by comparing the second to the first set of CRD at each distension pressure with a one-way ANOVA and Newman–Keuls post-test comparisons. When comparing the pICP response after repeated WAS in S-SH, NS-GH, and NS-SH mice, data were expressed as the mean change from baseline (Δ VMR in percentage control) for different pressures of distension and analyzed using two-way ANOVA and Bonferroni *post hoc* test. The 1-h time course of defecation compared with the first day in each group and comparison of the mean defecation over 10 days between groups were analyzed using one-way ANOVA followed by Newman–Keuls *post hoc* test, and daily defecation was compared between groups using a two-way ANOVA followed by Bonferroni *post hoc* test. Results are expressed as mean $\pm$ SEM, and P values < 0.05 were considered statistically significant.

Results

Validation of ICP as a measure of VMR to CRD in conscious mice

The inflation of the intracolonic balloon in a conscious mouse held in a restrainer induces an increased VMR captured by both EMG (Figure 1(B), top line) and ICP (Figure 1(B), second line from bottom) recordings within the first seconds of distension. The comparison of the ICP and EMG signals processed identically suggested that the pICP reflects the rapid contractile activity of the abdominal musculature. Both EMG and pICP signals also show a significant overlap indicating that they detect a similar CRD-related response linked to abdominal muscle contractions (Figure 1(B)). Both EMG and ICP signals induced by CRD display a polyphasic pattern characterized by bouts of strong activity; in particular at the onset and immediately after the inflation of the balloon and before deflation. Consequently, to avoid truncating the data, we analyzed the VMR over the whole period of distension as previously described (Cenac et al. 2007; Christianson and Gebhart 2007; Gecse et al. 2008). During graded phasic CRD, there was a strong linear correlation between the pressures of CRD and AUC values obtained by EMG ($n = 9$; $R^2 = 0.9441$; $p = 0.0284$) or ICP ($n = 9$; $R^2 = 0.9785$; $p = 0.0108$) recordings, and a comparable pressure-related increase (Figure 2(A)). At 60 mmHg, the EMG response was $96.2 \pm 3.3\%$ higher than at 15 mmHg ($P < 0.05$, $n = 9$), and the ICP response was increased by $87.7 \pm 9.5\%$ ($P < 0.05$ vs. 15 mmHg, $n = 9$; Figure 2(A)). Linear regression analysis showed a strong correlation between both measures during the 15–60 mmHg paradigm, when assessed simultaneously ($P < 0.05$, Figure 2(B)). In the same experiment, the threshold pressure to induce VMR in conscious mice was similar when assessed using the EMG vs. ICP method (31.4 ± 1.3 vs. 32.4 ± 2.2 mmHg, $n = 9$, respectively).

We further validated the ICP method in NS-GH mice by showing that a subcutaneous injection of atropine sulfate ($n = 10$) did not affect the VMR to the second CRD set compared to baseline [two-way ANOVA, $F(1,64) = 0.0214$, $P = 0.8848$](Figure 3), while buprenorphine injected i.p. ($n = 9$) significantly inhibited the VMR to the second CRD set compared to baseline [$F(7,71) = 13.53$, $P < 0.0001$] and saline [$F(7, 71) = 11.03$, $P < 0.0001$]. *Post hoc* comparison showed a significant decrease in VMR to CRD at 30, 45, and

60 mmHg compared to baseline and saline (Newman–Keuls *post hoc* test, $P < 0.05$, 0.01, and 0.01, respectively; Figure 4). Mice administered saline i.p. ($n = 10$) had similar VMR during the first and second sets of CRD (Figure 4).

Influence of surgery/SH on defecation to repeated WAS in conscious mice

There were no significant differences in weight gain between the different groups of mice S-SH ($n = 13$), NS-SH ($n = 10$ –13) or NS-GH ($n = 18$) exposed to daily sessions of WAS for 10 days (Figure 5(A)), or between groups of S-SH, NS-SH, or NS-GH mice kept in their home cages (data not shown). The mean basal pellets/h produced in mice kept in the home cage for the 10-day experimental period was not significantly different between NS-GH (2.8 ± 0.3 , $n = 4$), NS-SH (1.6 ± 0.2 , $n = 13$), and S-SH (3.1 ± 0.6 , $n = 5$) mice (Figure 5(B)) [$F(5, 65) = 10.61$, $P < 0.0001$] (Newman–Keuls *post hoc* test, $P > 0.05$ for each). WAS exposure 1 h/day for 10 days increased the mean defecation score over the 10 days, but this reached significance only in the S-SH (Newman–Keuls *post hoc* test, $P < 0.001$, $n = 13$) and not in the NS-SH (Newman–Keuls *post hoc* test, $P > 0.05$, $n = 13$) or NS-GH groups (Newman–Keuls *post hoc* test, $P > 0.05$, $n = 18$) when compared with the values in the respective S-SH, NS-SH, and NS-GH groups not exposed to stress (Figure 5(B)). The first 1-h exposure to WAS resulted in a significant increase in defecation in the NS-GH [$F(7, 370) = 7.074$, $P < 0.0001$] (Bonferroni *post hoc* test, $P < 0.001$) and S-SH mice [$F(9, 330) = 4.929$, $P < 0.0001$] (Bonferroni *post hoc* test, $P < 0.001$) compared with the respective NS-GH and S-SH groups left undisturbed in the home cage (Figure 5(C)). After repeated WAS, the defecation response in the NS-GH and S-SH groups decreased to reach basal values as in the NS-GH and S-SH groups left in the home cage, by days 3 and 6, respectively (Figure 5(C)). By contrast, NS-SH mice had no significant response to WAS throughout the experiment except on day 2 [$F(9, 320) = 2.807$, $P < 0.0001$] (Bonferroni *post hoc* test, $P < 0.001$).

Influence of surgery/SH on visceral sensitivity to repeated WAS in conscious mice

With regard to the VMR, the baseline response to first CRD in naïve mice before being assigned to S-SH ($n = 13$), NS-GH ($n = 18$), and NS-SH ($n = 10$) was similar in all groups. Repeated 1-h WAS for 10 days influenced differently the response to the second CRD performed 24 h after the last WAS session in the three groups when compared to their respective baseline (Figure 6(A)–(C)). Thus in the S-SH mice (Figure 6(A)), there was an increase in the VMR to CRD [$F(7, 103) = 19.80$, $P < 0.0001$], reaching significance at 60 mmHg (Newman–Keuls *post hoc* test, $P < 0.05$), while in NS-GH mice there was a significant decrease [$F(7, 143) = 27.99$, $P < 0.0001$] occurring at 30, 45, and 60 mmHg CRD pressures (Newman–Keuls *post hoc* test, $P < 0.05$, $P < 0.01$, and $P < 0.01$, respectively) (Figure 6(B)). These data contrast with the lack of change in NS-SH mice at all CRD pressures [$F(7, 78) = 8.492$, $P > 0.05$] (Figure 6(C)). Comparison of mean VMR changes from their baseline between the S-SH, NS-SH, and NS-GH groups 24-h post WAS showed that VMR to CRD at 30, 45, and 60 mmHg was significantly higher in S-SH compared to NS-GH mice (Bonferroni *post hoc* test, $P < 0.05$, $P < 0.05$, and $P < 0.01$, respectively), while there were no significant differences between NS-SH and NS-GH mice at all pressures of distension (Bonferroni *post hoc* test, $P > 0.05$) (Figure 6(D)).

Discussion

In the present study, using manometry to assess visceral pain, we were able to show that mice that underwent surgery for implantation of EMG electrodes and that were housed singly thereafter as part of the classical method of recording visceral sensitivity in rodents exhibited an increased VMR to CRD as monitored 24 h after the last session of repeated WAS. In contrast, group-housed mice not subjected to EMG surgery exhibited a decreased

visceral response to CRD 24 h after the last WAS stress period, while single-housed mice that did not undergo surgery exhibited no change in their visceral response. To monitor the VMR to CRD in conscious mice in a minimally invasive way, we have validated an alternative, surgery-free approach derived from a previous study (Arvidsson et al. 2006) using a modified commercially available miniaturized pressure catheter (solid-state manometry). Taken together, our data suggest that additional parameters linked to the methods and associated conditions used to measure viscerosensitivity in mice dramatically affect the pattern of visceral response to a repeated psychological stressor.

Classically, visceral pain in rodents is assessed by monitoring the VMR to CRD (Ness and Gebhart 1988) via recording the abdominal contractions through EMG signals captured by electrodes chronically implanted in the abdominal musculature (Kamp et al. 2003; Larsson et al. 2003; Cenac et al. 2007; Christianson and Gebhart 2007; Verma-Gandhu et al. 2007). Although the method is of significant value in the field of visceral pain study, it has experimental inconveniences such as damage to EMG electrodes, loss of signal, and electrical interference. This is particularly true in chronic experimental settings. The present data show that ICP measurements have similar sensitivity and reproducibility as EMG recordings in conscious mice. This is supported by the correlation between signals from ICP and EMG during consecutive ascending phasic distensions between 15 to 60 mmHg when recorded simultaneously in the same mice. We also showed that the colonic pain pressure threshold to CRD detected by both methods is similar (about 32 mmHg), and consistent with values reported using EMG and manometry (Kamp et al. 2003; Arvidsson et al. 2006). We also found that both EMG and ICP signals displayed a polyphasic response characterized by bouts of strong activity; in particular at the onset and immediately after inflation of the balloon and before deflation, which led us to consider the integral of 10-s responses to CRD for analysis. A similar pattern of multi-bout response has been reported in several other studies (Cenac et al. 2007; Christianson and Gebhart 2007; Geese et al. 2008). However, there is also evidence that CRD at 80 mmHg for 10 s results in a single bout pattern of contraction (Arvidsson et al. 2006). Whether such a difference relates to the experimental conditions (age, sex of mice, or intensity of CRD pressure) is unknown. The bouts of activity are not related to artifacts due to balloon interference to the pressure sensor, because they were also present in the EMG signal far from the balloon or pressure sensor when both activity changes to CRD were recorded simultaneously in conscious mice. A similar phenomenon has also been described in human during rectal distension where pain ratings were found to increase significantly at the beginning of the inflation and also during deflation (Kwan et al. 2002). In addition, as colonic pressure could be altered following abdominal contractions and/or contractions of the colonic wall proper, we further validated the ability of the ICP method to discriminate colonic pressure changes induced by abdominal vs. colonic wall contractions and to detect analgesic effects using specific data analysis scripts and pharmacological tools. Two sets of evidence rule out that contractions originating from the colonic smooth muscles affect the ICP signal during CRD. First, as previously reported with EMG and manometry recordings in conscious mice (Arvidsson et al. 2006), the peripheral injection of the muscarinic receptor antagonist, atropine sulfate, did not affect phasic CRD-associated ICP changes while inhibiting distal colonic motility assessed by ICP in conscious mice maintained under similar recording conditions (Gourcerol et al. 2009). Second, the parameters used in the script of analysis in Spike 2 were chosen such that only fast activity events of less than 2-s duration (i.e. abdominal contractions) were selected for the analysis. Smooth muscle contraction and somatic muscle contraction (e.g. the external obliques) indeed occur with different time constants. For example, typical “phasic” contraction of smooth muscle lasted 25–30 s in isolated mouse colon (Fida et al. 1997; Bush et al. 2000; Powell and Bywater 2001) and 16.4 ± 0.69 s in conscious mice (Gourcerol et al. 2009). Last, buprenorphine, a partial agonist for μ -opioid receptors (Kamei et al. 1995; Christoph et al. 2005), inhibited the visceral sensitivity to

graded phasic ascending CRD consistent with reported reduction of the basal visceral response to CRD by opioid analgesics in both rats and mice (Danzebrink et al. 1995; Larsson et al. 2003). Taken together, these data established the reliability of the ICP method under our conditions to detect modulation of visceral sensitivity in conscious mice.

The present validation of ICP recording (solid-state manometry) as a non-invasive method to assess VMR to CRD in conscious mice extends earlier reports on visceral pain assessment in conscious mice using a manometric approach (Arvidsson et al. 2006). Although direct comparison between the previously described manometric technique and ours could not be made, the present comparison with the reference technique of EMG recordings suggests that both techniques are equally sensitive as they detect similar pain thresholds (~32 mmHg) and have a similar correlation between EMG and manometry signals, with values of $R^2 = 0.862$ in our study vs. $R^2 = 0.899$ previously reported (Arvidsson et al. 2006). There are, however, two main differences between the two manometric techniques. The first one lies in the use of different equipment and approach to measure a similar parameter, which is the ICP. The previous studies (Arvidsson et al. 2006; Martinez and Melgar 2008; Brusberg et al. 2009; Larsson et al. 2009) used a distension balloon and assessed the changes in pressure inside the balloon due to abdominal contractions via a pressure sensor interfaced between the balloon and the barostat. In the current study, a miniaturized pressure catheter was directly inserted inside the colon cavity to detect the luminal pressure from within—which varies depending on the contraction of the abdominal wall—as an indirect measure of abdominal muscle contraction. The second difference comes from the easiness of the procedure, which relies on using a pressure catheter, software, and barostat that are commercially available compared with the previously described setups which for the most part (barostat and software) are custom made and therefore not readily commercially available (Arvidsson et al. 2006).

WAS is a common psychological stressor used in rats (Bonaz and Taché 1994; Miampamba et al. 2007; Larauche et al. 2008) which has been applied recently in mice (Cameron and Perdue 2005; Melgar et al. 2008; Larsson et al. 2009). The present data show that 24 h after the last exposure to WAS repeated for 10 days, 1 h/day, singly housed male mice with chronic EMG implantation surgery exhibit a visceral hypersensitivity to CRD assessed by ICP. By contrast, there was a significant reduction in VMR to CRD at all pressures of distension in male mice that were group housed and had no previous surgery, when assessed 24 h after the last WAS session, suggesting the development of stress-related analgesia in these mice. Interestingly, mice without previous surgery but single housed similarly to S-SH mice throughout the experiment did not show any reduction in their VMR compared to baseline 24 h after the end of repeated WAS. Likewise, earlier studies in mice showed that previous social isolation stress, compared to group housing, reduces the somatic analgesia response to a subsequent acute restraint stress, which has been suggested to involve a decreased sensitivity of opioid receptors to opiate level since the analgesic response to morphine was abolished in individually housed mice (Puglisi-Allegra and Oliverio 1983). It is noteworthy that in our experiment SH was performed under conditions of an enriched environment, including the presence of nest material that may minimize the impact of such housing conditions. Other studies have shown in rats equipped with chronic abdominal EMG electrodes implanted surgically that acute WAS induces delayed visceral hypersensitivity to CRD, 24 h after a first session of stress (Schwetz et al. 2004) as well as a state of maintained visceral hyperalgesia that lasts over 40 days upon repeated WAS exposure (10 days, 1 h/day) (Bradesi et al. 2005; Hong et al. 2009). In contrast, a recent report indicates that repeated WAS for 10 days has no effect on visceral pain to CRD in group-housed female mice, as monitored by manometry at the end or at 24 h after the last stress session of repeated WAS for 10 days (Larsson et al. 2009). As there is evidence that male mice display higher WAS-induced visceral analgesia than female mice (Gui et al.

2004), the recruitment of this pathway may be consistent with the WAS-induced decrease in VMR to CRD observed in male mice in the present study while having no effect in female mice (Larsson et al. 2009), under conditions of manometry recording in group-housed mice. In addition, repeated homotypic psychological stress can also induce changes in endocannabinoid tone (Patel and Hillard 2008; Hong et al. 2009), and recent data suggest role of endocannabinoids and their receptors in rat and mouse visceral sensitivity (Brusberg et al. 2009; Naidu et al. 2009). In contrast, the sensitized background of S-SH mice linked to their social isolation status and surgery encompassing a permanently implanted device could represent a subthreshold inflammatory stimulus necessary to reveal the interaction between stress and hypersensitivity. In agreement with this hypothesis, we observed that S-SH and NS-GH mice had a strong similar defecation response to the first WAS session; however, the return to baseline defecation values observed in mice not exposed to WAS occurred on day 3 in NS-GH mice and on day 6 in the S-SH group. Interestingly, NS-SH mice did not show much defecation in response to the first exposure to WAS, indicating that some of the effects of WAS exposure linked to novel environment and/or social isolation may have been affected by the previous period of SH. The assessment of the defecation response is a quantitative parameter of propulsive colonic motor functions induced by stress (Bonaz and Taché 1994; Taché and Bonaz 2007). The distinct temporal patterns of defecation in response to WAS for 10 days in NS-GH, NS-SH, and S-SH mice indicate that NS-SH mice had little responsiveness to repeated WAS, and that NS-GH mice became habituated to the repeated exposure to the WAS within 3 days, while there was a delay in the habituation to this homotypic stressor in S-SH mice. This may reflect the greater intensity of stress under these combined conditions of repeated WAS plus SH and surgery. Taken together, these data indicate that combined factors such as isolation, surgery, and related conditions of VMR recording using chronically implanted EMG electrodes are necessary components for the visceral hypersensitivity observed 24 h after the end of 1 h daily exposure to WAS for 10 days and for the continued defecation response to each WAS exposure for 7 days. Further studies are required to directly address the underlying mechanisms related to the development of homotypic stress-induced analgesia and its reversal to hyperalgesia in mice with chronic surgical implantation of EMG electrodes and related SH.

In summary, the present results provide the first evidence that the combination of conditions associated with the traditional VMR measurement, consisting of surgery together with chronically implanted abdominal electrodes and associated post-surgical SH, impacts on the visceral response of mice to a subsequent intermittent daily exposure to a psychological stressor. We showed that the visceral responses (defecation and VMR) to repeated WAS are heightened in socially isolated mice that have undergone previous surgery and chronic device implantation to monitor VMR, compared with group-housed or single-housed mice with no previous surgery and implantation of EMG electrodes. This raises the need to reassess classical methods to monitor visceral pain in mice and the careful consideration of housing context (single or grouped) on this parameter. In addition, we validated the feasibility and reliability of ICP measurement (solid-state manometry) to monitor visceral pain in conscious mice and proposed a commercially available alternative method that can be readily implemented in research laboratories.

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Figure 1.

Example traces of simultaneous recording of visceral motor response (VMR) to CRD by electromyography (EMG) and intraluminal colonic pressure (ICP) in conscious mice. (A) Schema of the modified miniaturized pressure catheter equipped with the custom-made polyethylene plastic balloon to measure ICP. The 2 cm-long polyethylene balloon is ligated 1 cm below the pressure probe. (B) Original and rectified representative EMG and ICP traces recorded simultaneously from the same mouse in response to CRD (45 mmHg, 10 s). When both raw EMG (upper line) and ICP (second line from the bottom) signals are analyzed in Spike 2 by computing “DC Remove” 1 s to exclude all slow events >2 s (i.e. colonic smooth muscle contractions) and “root mean square (RMS) amplitude” to extract the

area AUC of the signal, the resulting EMG and phasic ICP (pICP) signals (middle lines) show an obvious similarity in waveform. (C) Representative EMG and ICP traces at 45 and 60 mmHg CRDs. The VMR is polyphasic, covering the whole distension period with bouts of activity at the beginning and end of the distension period; hence VMR was measured over the 10-s distension.

Figure 2.

(A) Ascending phasic CRD results in similar VMR changes as monitored simultaneously by EMG (filled squares) and ICP (open circles) in conscious mice equipped with EMG electrodes in the abdominal wall and single housed for 5 days post surgery. Broken lines represent the linear correlation curves $p = 0.0284$ for EMG and $p = 0.0108$ for ICP. (B) Correlation between EMG and ICP responses to ascending phasic CRD in mice. Broken lines represent the 95% confidence intervals, dotted line represents the baseline. Data are expressed as mean $\pm$ SEM of AUC from $n = 9$ mice.

Figure 3.

The muscarinic receptor antagonist, atropine did not alter the VMR to CRD monitored with ICP in naïve mice. A first set of ascending phasic CRD was performed in 10 mice and after 45 min, atropine (1 mg/kg) was injected subcutaneously and 15 min later, a second CRD was performed. Data are expressed as mean $\pm$ SEM of VMR expressed as % of 60 mmHg response to the first CRD.

Figure 4.

The analgesic buprenorphine inhibited the VMR to ascending CRD monitored with ICP in naïve mice. A first set of ascending phasic CRD was performed and 45 min later, buprenorphine (0.3 mg/kg) or saline was injected i.p. and after 15 min, a second CRD was performed. Data are expressed as mean $\pm$ SEM, of 9–10 mice per group. * $P < 0.05$ compared with baseline (pooled groups of the first CRD) or i.p. vehicle.

Figure 5.

Influence of repeated intermittent WAS on BW and defecation in mice subjected to EMG electrode implantation and SH post surgery (S-SH) compared with mice having no surgery and group-housed (NS-GH) or having no surgery and single-housed (NS-SH). (A) Daily BW changes in S-SH, NS-SH and NS-GH mice exposed daily to 1 h of WAS for 10 days. Each point represents the mean $\pm$ SEM for $n = 13$ –18 mice. (B) S-SH mice exhibit an increased average defecation in response to repeated WAS compared to NS-GH and NS-SH mice. In contrast, S-SH, NS-GH and NS-SH mice when monitored in non-stressful conditions (basal non-stressful defecation in home cage for 1 h following a quick bedding change) exhibit similar defecation rates. Each bar represents the mean $\pm$ SEM of number of mice indicated in the bars; * $P < 0.05$ vs. S-SH control group, + $P < 0.05$ vs. all other groups. (C) Time course of defecation response to 1-h WAS in NS-GH, S-SH and NS-SH mice. Each point represents the mean $\pm$ SEM for $n = 13$ –18 mice. The grey band represents the mean $\pm$ SEM basal defecation of mice under non-stressful conditions during 10 days (pooled values of NS-GH, $n = 4$; S-SH, $n = 5$ and NS-SH, $n = 13$). * $P < 0.05$ vs. FPO at day 1 for respective groups, # $P < 0.05$ vs. mean basal non-stressful defecation, + $P < 0.05$ compared with NS-GH + WAS group, \$ $P < 0.05$ vs. NS-SH + WAS group.

Figure 6.

Repeated WAS alters differently the visceral sensitivity to CRD in S-SH, NS-GH, or NS-SH mice. (A) S-SH mice exhibit an increased VMR to CRD at 60 mmHg compared to baseline. (B) NS-GH mice exhibit a decreased VMR to CRD at 30, 45, and 60 mmHg compared to baseline. (C) NS-SH mice show no significant changes in VMR post WAS compared to baseline. (D) S-SH mice exhibit an increased VMR to CRD at 30, 45, and 60 mmHg vs. NS-GH mice as monitored 24 h after the last session of repeated WAS (1 h/day for 10 days). Data are expressed as mean $\pm$ SEM, $n = 10$ –18 per group as given in graphs. * $P < 0.05$ vs. baseline, + $P < 0.05$ vs. S-SH mice.