

RESEARCH ARTICLE

Regional brain activation during rectal distention and attenuation with alosetron in a nonhuman primate model of irritable bowel syndrome

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Abstract

Greater understanding of the mechanism that mediates visceral pain and hypersensitivity associated with irritable bowel syndrome (IBS) would facilitate the development of effective therapeutics to manage these symptoms. An objective marker associated with the underlying mechanisms of visceral pain and hypersensitivity could be used to guide therapeutic development. The current study examined brain activation evoked by rectal distention with functional magnetic resonance imaging (fMRI) in a cynomolgus macaque model of visceral hypersensitivity. Male, cynomolgus macaques underwent five four-week treatments of dextran sodium sulfate (DSS)-distilled water (DW), which induced mild-moderate colitis with remission during each treatment cycle. Balloon rectal distention (RD) was performed under anesthesia 14 weeks after the final DSS-DW treatment. Colonoscopy confirmed the absence of colitis prior to the start of RD. In naïve, untreated macaques, 10, 20 and 30 ml RD did not evoke brain activation. However, insular cortex/somatosensory II cortex and cerebellum were significantly activated in DSS-treated macaques at 20 and 30 ml rectal distention. Intra-rectal pressure after DSS treatment was not significantly different from that of naïve, untreated macaques, indicating lack of alteration of rectal functioning following DSS-treatment. Treatment with 5-HT₃ receptor antagonist alosetron (p.o.) reduced distention-evoked brain activation and decreased intra-rectal pressure. The current findings demonstrated activation of brain regions to RD following DSS treatments which was not present in naïve macaques, suggesting visceral hypersensitivity. Brain activation in turn was reduced by alosetron, which could underlie the analgesic effect alosetron in IBS patients.

Abbreviations: CC, cingulate cortex; DSS, dextran sulfate sodium; EI, Endoscopic Index; fMRI, functional magnetic resonance imaging; Ins/SII, insular cortex and secondary somatosensory cortex; IBS, irritable bowel syndrome; RD, rectal distention; UC, ulcerative colitis.

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KEYWORDS

cingulate cortex, insular/secondary somatosensory cortex; neuroimaging; translational, *Macaca fascicularis*, manometry, visceral hypersensitivity

1 | INTRODUCTION

One of the key symptoms of irritable bowel syndrome (IBS) is abdominal pain and visceral hypersensitivity—most (40%–60%) IBS patients demonstrate decreased thresholds, compared to healthy controls, to acute phasic rectal distension (RD).¹ Irritable bowel syndrome has been suggested to be a brain-gut-microbiota axis dysfunction yet thorough understanding of the linkage between the components and their relationship to pain and visceral hypersensitivity is lacking.^{1–3} While a number of preclinical studies have identified potential molecular targets associated with visceral pain and hypersensitivity, these molecular targets have not been entirely validated in clinical IBS trials.^{4,5} There are few therapeutics available that directly address pain and hypersensitivity associated with IBS.²

Most of the current knowledge concerning the basic mechanism of pain and hypersensitivity associated with IBS have been derived from preclinical studies based on rodent models.^{1,4} While rodents are a convenient nonhuman species to model clinical disease, there are significant differences between rodent and human immunological functioning, neuroanatomy and neurological functioning, such that findings in rodents could lead to erroneous conceptions of clinical pathophysiology.^{6–9} Furthermore, the ability of current nonhuman animal models to predict efficacy in humans of a new treatment based on a novel therapeutic mechanism of action, that is, predictive value, has been “disappointing”, which raises the question of the translatability of findings from rodent models of IBS to clinical IBS, particularly pain and hypersensitivity.^{10–15}

One of the potential barriers to successful clinical translation from laboratory findings is the lack of an objective biomarker for pain and hypersensitivity associated with IBS as pain perception is a confluence of affective, cognitive, motor as well as sensory processes and the contribution of each component could differ between patients.^{4,16} Irritable bowel syndrome patients report pain following colorectal distension at pressures and volumes that are non-painful in healthy controls yet the primary outcome measure is individual description of pain intensity.^{9,12,17} Also, the intervention's primary mechanism of action may be not be directly related to the improvement in clinical symptoms. Thus, an outcome measure, a biomarker, that is more objective than self-rating and related

to the underlying pathophysiology would aid not only in elaborating mechanism but also indicate relevance of the target to the therapeutic's mechanism of action.¹⁶

Regional brain activation in IBS patients evoked by RD has been reported and could serve as a non-subjective marker of visceral pain and hypersensitivity.^{16,18} Rectal distention-evoked regions include the cerebellum, cingulate cortex (CC) and insular cortex (Ins).¹⁹ Similarly activated brain regions have been reported in a stress-induced rat model of visceral hypersensitivity.²⁰ Whether brain activation occurs in other nonhuman animal models of IBS has yet to be reported.²¹ In addition, the effects of clinical therapeutics on brain activation in these nonhuman animal models have yet to be examination.

A nonhuman primate model of visceral hypersensitivity following treatment with dextran sulfate sodium (DSS) was developed in the current study. Activation of brain regions associated with pain during RD was utilized as the primary outcome measure. The cynomolgus macaque immunologic response to pathogens, gut microbiome and gut functioning are closer to humans than rodents.^{6,8,22–24} In addition, it is possible to induce experimentally induced ulcerative colitis in the macaque over a greater period of time and with greater frequency than in rodents.^{25–27} Thus, regional brain activation observed in the macaque model could serve as the nonhuman animal equivalent of brain activation observed during rectal distention in IBS patients. Activation of key brain regions associated with pain following rectal distention in DSS-treated macaques but not in naïve macaques suggests that the macaque model could be used to explore the central mechanism of pain associated with IBS and test novel therapeutic interventions.

Alosetron is a 5-HT₃ receptor antagonist that is approved for the management of painful symptoms associated with IBS (albeit for patients under certain conditions due to serious adverse events).²⁸ The mechanism of action of alosetron has been speculated to be both peripherally and centrally mediated, by slowing colonic transit time and by reducing activation of brain regions associated with pain perception.²⁸ Serotonin type-3 (5-HT₃) receptors have been found in colonic mucosal and muscularis tissue as well as autonomic and sensory nerves and brain.^{29,30} Thus, alosetron was used to determine if evoked brain activation in the DSS-treated macaques was sensitive to a drug that is used to manage IBS symptoms.

2 | MATERIALS AND METHODS

2.1 | Subjects

A total of eight male *Macaca fascicularis* (approximately 5 years old and 4.1–5.8 kg body weight range at the end of the study; Eve Bioscience Co.) were used in the current study. Of the eight macaques, four underwent repeated DSS-distilled water (DW) cycles and four were not treated with DSS-DW. Procedures involving macaques were reviewed and approved by the Hamamatsu Pharma Research Animal Care and Use Committee (Approval no. HSTIRB-284). Environmental management and housing conditions were according to the *Guide for the Care and Use of Laboratory Animals, Eighth Ed.*³¹ and the housing facility is fully accredited by AAALAC International. Room temperature and humidity were continuously monitored and adjusted when necessary to set ranges. During the course of the study, macaques were individually housed in order to monitor individual fluid intake and to monitor individual health parameters. Macaques retained auditory, visual and olfactory contact with conspecifics and were provided with manipulanda. Throughout treatment, macaques were fed once daily a standard nonhuman primate diet (Oriental Yeast Co., Ltd.) and were hand-fed either study staff or animal care staff fresh fruit or vegetables at least once per week. In case a macaque showed a sudden, acute loss of $\geq 25\%$ of body weight, diminished feeding, lethargy and unresponsiveness to stimuli, DSS treatment was to be discontinued and the macaque was removed from the study for treatment. In the current study, all four DSS-treated macaques showed normal weight gain and displayed no signs of extreme pain or distress.

Rectal distention and MRI were not performed in macaques before the first cycle of DSS and distilled water (DW) treatment. Thus, four naïve, untreated macaques, similar in age and weight to macaques that underwent DSS and DW treatments and RD and MRI, were used to examine “baseline” RD and MRI.

At the end of the current study, four DSS-treated macaques were euthanized with secobarbital (100 mg/kg, i.v. Nichi-Iko Pharmaceutical) for tissue collection, unrelated to the current study. The four naïve, untreated macaques were returned to the colony.

2.2 | DSS-induced intermittent colitis

Figure 1 shows a time-line outlining the current study.

Dextran sulfate sodium-treated macaques were used from a previous study.²⁷ The macaques underwent a total of five treatment cycles of DSS followed by DW without DSS (Figure 1).³² One treatment cycle consisted of 14 days of DSS followed by 14 days of DW without DSS. Dextran sulfate sodium (DSS; 0.25% [w/v]; M.W. 36,000–50,000; MP Biomedicals) was dissolved in distilled water (DW) mixed with Kool-Aid® Invisible Drink Mix (The Kraft Heinz Company) and individual macaques were allowed free access to DSS supplied in a bottle attached to each cage. Daily total intake of DDS was 100 ml/kg.

On the afternoon before colonoscopy, fMRI and rectal distention measurements, macaques were given laxatives (0.75% picosulfate solution [Nichiiko] and 14% magnesium citrate [Horii Pharmaceutical] in water). Food for these macaques were withheld starting on the evening before the day of these procedures.

2.3 | Semi-quantitative measurement of disease activity

Disease activity was assessed using a modified Mayo score before administration of DSS and at regular intervals following DSS and DW administration.²⁷ The modified Mayo score consisted of: stool consistency (modified Bristol stool scale³³), rectal bleeding, colonoscopy examination³⁴ and “global assessment,” or general assessment of health. Subscores ranged from 0–3, with 3 being severe. The worst stool consistency and rectal bleeding from within 3 days of examination was used.

“Global assessment” was modified (Table S1), from a previous description, which now included: body weight, activity, appetite, and drinking volume.²⁷ Global assessment scores now ranged from 0–9, with 9 being severe signs of illness. Body weight assessment for establishing a Mayo score was performed once. Scoring of body weight for the purpose of establishing a Mayo score was modified from the previous scoring system: no change or an increase in body weight, 0; change of less than 5% (either increase or decrease), 2; change of less than

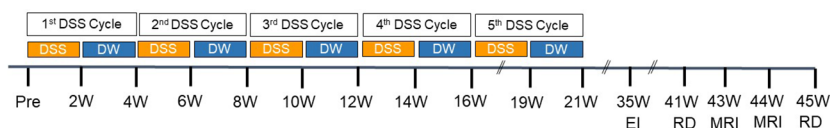


FIGURE 1 Study time course. Dextran sulfate sodium (DSS) and distilled water (DW)-treated macaques from a previous study were evaluated in the current study.²⁷ Fourteen weeks after the last DSS and DW treatment cycle, macaques were scored with colonoscopy (endoscopic index, EI) and Mayo score to confirm remission from inflammation. Rectal distention (RD) and examination of RD-evoked brain activation with fMRI (MRI) were performed no more than once per week.

10% (either increase or decrease), 4; change of greater than 10% (either increase or decrease), 6; Changes in appetite and drinking were non-quantified observations of changes in eating or drinking (or a lack of eating or drinking) from the previous day. Global assessment score was then converted to a score ranging from “0” to “3”: 0 to 2 converted to “0”; 3 and 4 converted to “1”; 5 through 7 converted to “2” and 8 and 9 converted to “3”. In the case of global assessment, unlike the case in the clinical setting, wherein results from the other subscores are “acknowledged” in the final global assessment score, results from the other subscales did not influence final scoring.³⁵ An endoscopic examination needed to establish a Mayo score was performed once.

A Rachmilewitz Endoscopic Index (EI) was used to score endoscopic findings, which was scaled differently from the Mayo colonoscopy examination score.³⁶ Scores from the four subcategories ranged from 0–4, with 4 being severe and a composite was reported, ranging from 0–12, with 12 being the most severe.²⁷

2.4 | Intra-rectal pressure measurement

Measurement of intra-rectal pressure (mm Hg) was performed to observe changes in rectal compliance (change in intra-rectal pressure related to changing volume of air) after induction of DSS and water treatment.

Under propofol anesthesia (0.2 mg/kg/h; Maruishi Pharmaceutical), macaques underwent a block design rectal stimulation protocol similar to that of Wang et al.¹⁹ A Sengstaken-Blakemore (S-B) tube (Type 52; Create Medic Co.) was placed and fixed at 5 cm from the anus. The maximum inflation volume of the (gastric) balloon was 400 ml. Balloon distention with air via a syringe was set to 10, 20, and 30 ml (Figure 2). Thirty seconds of no stimulus was followed by 30 s of balloon distention, followed by 30 s of rest and this sequence was repeated three times for each volume.

The S-B tube valve was attached to a blood pressure amplifier (AP-641G, Nihon Kohden Co., Ltd.) via a blood pressure transducer (DX-100, Nihon Kohden Co., Ltd.) and intra-rectal pressure was plotted (WR300, Graphtec Co., Ltd.). The intra-rectal pressures were measured every 5 s. From the start of balloon inflation, and the results at each volume were expressed as an average of three measurements. When the peak intra-rectal pressure was obtained within 5 s from the start of inflation, maximum pressure was set at this value.

The average (\pm SD) pressure (mm Hg) exerted by 10, 20 and 30 ml of air was 12.8 ± 2.2 , 31.2 ± 4.1 and 60.3 ± 9.6 mm Hg, respectively—60.3 mm Hg is clearly within the range of noxious in humans (e.g., 40–60 mm Hg).¹⁷ At 15 and 30 mm Hg,

both IBS and healthy controls report “light pressure” and “stool or pressure”.³⁷

Intrarectal pressure measurements were performed no more than once per week.

2.5 | Functional magnetic resonance imaging (fMRI)

Functional images were obtained by using a Signa HDxt 3.0 T (GE Healthcare). Macaques were sedated during each scan by infusion of a non-analgesic dose of propofol (0.2 mg/kg/h; Maruishi Pharmaceutical).^{38–40} Heads were fixed with an MR compatible acrylic head holder (Matsui Co.). During the scan, macaques were kept warm with blankets and heating pads. Macaques were scanned no more than once per week.

During one fMRI scan, macaques underwent a block design rectal stimulation protocol as described earlier (Figure 2). One complete scan was about 20–30 min in duration.

The anatomical MRI protocol consisted of a T1-weighted fast spoiled gradient-recalled (FSPGR) sequence (repetition time (TR)/echo time (TE), 15.8/7.0 ms; number of averages, 1; flip angle, 12°; field of view, 150 mm \times 150 mm; matrix, 256 \times 224; slice thickness/interval, 1.0/0.5 mm; number of slices, 168). Functional scan sequences consisted of field-echo, echo-planar imaging (TR/TE, 3000/35 ms; flip angle, 90°; field of view, 140 mm \times 140 mm; matrix, 64 \times 64; slice thickness, 2.4 mm; number of slices, 30).

During one fMRI scan, animals underwent a block design stimulation protocol: 3 sets of mechanical stimulations using balloon distention with air was set to 10, 20, and 30 ml. One stimulation set consisted of 30 s of an “OFF” stimulus, balloon deflation, followed by 30 s of an “ON” stimulus, a 10, 20, and 30 ml balloon distention. For each set, 10 frames were acquired, for a total of 60 frames per functional scan. A 30 s Interval without stimulation separated each set. The balloon inflation volumes were tested in ascending order.

The fMRI data were analyzed using SPM12 (Wellcome Department of Cognitive Neurology). The images were realigned and resliced onto the mean echo planner imaging (EPI) image to correct for head motion, and normalized to a macaque brain template (Stereotaxic coordinates according to Horsley-Clarke's stereotaxic coordinates). The brain activation area was determined by SPM12, and was displayed superimposed on the 3D acquired T1-weighted image. The EPI images were superimposed to corresponding T1-weighted anatomical image, and normalized to a macaque brain template. The resulting image was smoothed with a 4 mm \times 4 mm \times 4 mm full-width a half-maximum Gaussian kernel. Voxel-wise statistical analysis was based on a general linear model.

A fixed-effect model was used for group analysis of data of four macaques. Contrast mapping was defined to isolate regions responsive to each volume of balloon distention stimulation-related signals in the whole brain. Peak voxels statistical analysis was performed based on a general linear model (uncorrected for multiple comparison, one-tailed *t*-test). Peak voxel *z* scores greater than 1.96 were considered statistically significant at $p < 0.05$.

2.6 | Alosetron treatment

Alosetron HCl (Sigma-Aldrich Inc.) was orally administered at 0.1 mg/kg (free base) approximately 1 h prior to fMRI and intra-rectal pressure measurement. In humans, the time to peak alosetron in plasma following oral administration is about 1 h.⁴¹ Alosetron was administered in a vehicle of 0.5% methyl cellulose (FUJIFILM Wako Pure Chemicals Corp.) in water.

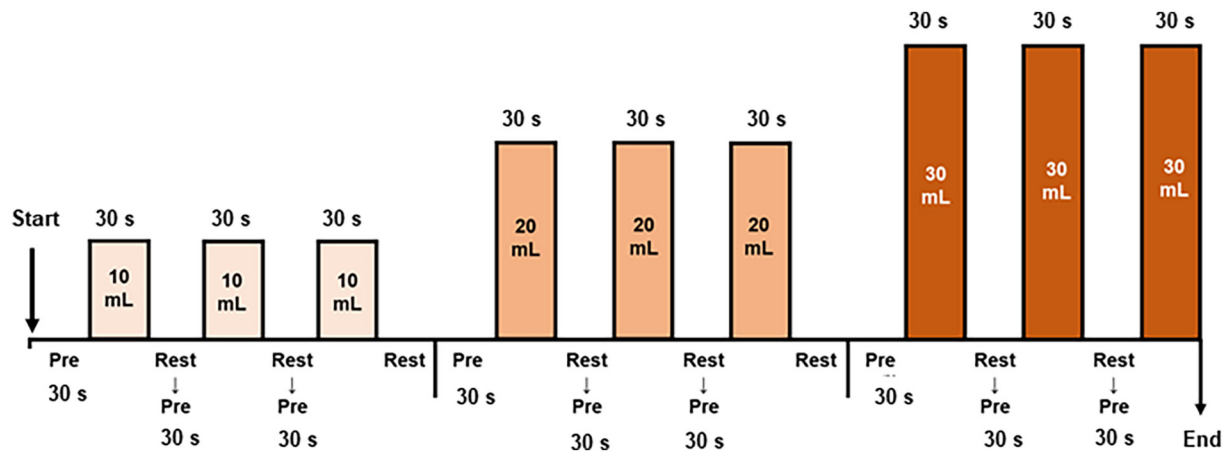


FIGURE 2 Balloon rectal distention stimulation sequence. Stimulation was performed under propofol anesthesia. Each stimulus was preceded by 30 s of rest (no stimulation and deflated balloon) and each stimulus was followed by 30 s of rest. Stimulation at each volume of air was for 30 s. At the end the final 30 ml stimulus, the balloon was removed from the macaque and allowed to recover from anesthesia.

Region/Volume	Hemisphere	Z score	Coordinates (mm)		
			<i>x</i>	<i>y</i>	<i>z</i>
<i>10 ml</i>					
Insula	Left	0.97	-16	20	2
	Right	0.92	14	18	2
Cingulate cortex		1.17	0	-2	14
Cerebellum	Right	0.91	-4	-8	-10
<i>20 ml</i>					
Insula	Left	1.28	-16	20	2
	Right	1.30	14	18	2
Cingulate cortex		1.33	0	-2	12
Cerebellum	Right	1.23	-4	-8	-10
<i>30 ml</i>					
Insula	Left	1.77	-16	20	2
	Right	1.82	14	18	2
Cingulate cortex		1.52	0	-2	12
Cerebellum	Right	1.58	-4	-8	-10

TABLE 1 Lack of activation during rectal distention in naïve, untreated macaques

Note: Group mean peak voxels of naïve, untreated macaques during 10, 20 and 30 ml rectal balloon distention. Mean calculated from four macaques. No significant activation was observed at any rectal distention volume; mean *z* score less than 1.96, $p > 0.05$. Stereotaxic coordinates (*x*, *y*, *z*) are according to Horsley-Clarke's stereotaxic coordinates.

2.7 | Statistical analysis

Previous studies have utilized five to six macaques to demonstrate marked inflammation following DSS treatment in the gut and distal tissues and significant changes in colonic tissue protein expression, compared to untreated macaques.^{32,42} The least number of animals possible were used in the current study. To reduce the number of macaques needed for the current study, all four DSS-treated macaques underwent alosetron treatment and comparisons of the effect of alosetron treatment was made between before and then after alosetron administration in DSS-treated macaques.

Statistical analyses were performed using SAS Analytical Pro version 9.4 (SAS Institute Japan) and EXSUS version 10.1 (EP Croit Corp.). Data are expressed as mean \pm standard deviation (S.D.). Differences between treatments were analyzed with Student's *t*-test. Between group intra-rectal pressures over time was examined via

repeated measures two-way analysis of variance. *p* values less than 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Signs of ulcerative colitis following DSS and DW treatment

Within each DSS and DW treatment cycle, after each DSS treatment period, diarrhea and bloody stool were observed in all macaques.²⁷ At the same time, erythema, decreased or absent of vascular pattern, edema, bleeding, erosion and ulceration of the epithelium were observed with colonoscopy in the descending and sigmoidal colon. Remission of signs of disease was observed following DW treatment within each DSS and DW treatment cycle (Table S2).²⁷

At week 35 (about 14 weeks after the end of the DSS final cycle), Mayo score and EI were 0 in all DSS-treated

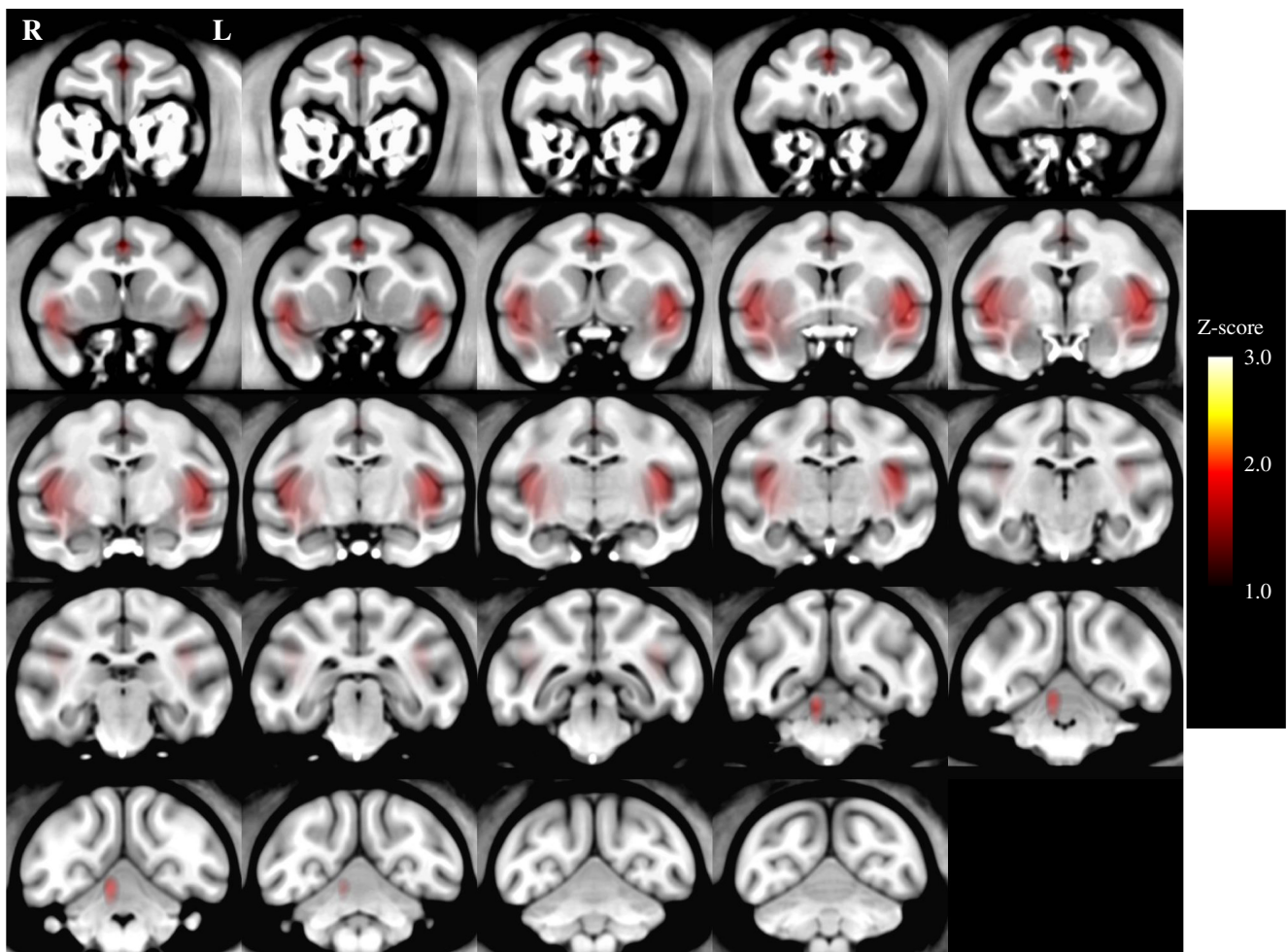


FIGURE 3 Coronal brain sections showing mean peak voxels during 30 ml rectal distention in naïve, untreated macaques. No significant activation was observed in naïve macaques ($n = 4$) at any balloon volume. Serial sections from rostral (upper left corner) to caudal (bottom right corner). R, macaque's right. L, macaque's left.

Region/Volume	Hemisphere	Z score	Coordinates (mm)		
			x	y	z
<i>10 ml</i>					
Insula	Left	1.39	-16	20	2
	Right	1.51	14	18	2
Cingulate cortex		1.41	0	-2	14
Cerebellum	Right	1.48	-4	-8	-10
<i>20 ml</i>					
Insula	Left	2.51	-16	20	2
	Right	2.59	14	18	2
Cingulate cortex		1.56	0	-2	12
Cerebellum	Right	2.14	-4	-8	-10
<i>30 ml</i>					
Insula	Left	3.09	-16	20	2
	Right	3.18	14	18	2
Cingulate cortex		1.79	0	-2	12
Cerebellum	Right	2.54	-4	-8	-10

Note: Group mean peak voxels of DSS-treated macaques during 10, 20 and 30 ml rectal balloon distention. Mean calculated from four macaques. Significant bilateral activation of insula and the right cerebellum was observed during 20 ml and 30 ml rectal distention. Stereotaxic coordinates (x, y, z) are according to Horsley-Clarke's stereotaxic coordinates. Mean z score greater than 1.96, $p < 0.05$. Mean z score greater than 2.58, $p < 0.01$.

macaques—signs of active inflammation were absent (Figure 1).

3.2 | Rectal distension-induced regional brain activation following DSS and DW treatment

In naïve, untreated macaques, no significant mean peak voxels were observed during RD (Table 1; Figure 3). By contrast, 14 weeks after the final DSS and DW treatment cycle, mean peak voxels were observed during 20 and 30 ml rectal distension bilaterally in insular cortex/secondary somatosensory cortex (Ins/SII) and cerebellum (Cb) (Table 2, Figure 4). (In one DSS-treated macaque (out of four), peak voxel was observed in the cingulate cortex (CC) with 20 and 30 ml rectal distention.)

3.3 | Effect of alosetrone on regional brain activation in DSS-treated macaques

Alosetron, administered p.o. 1 h prior to fMRI, suppressed emergence of distention-evoked brain activation at 20 ml balloon volume (Table 3). At 30 ml balloon volume, mean peak voxel was observed in the left insula (Table 3, Figure 5). Contrast subtraction between before alosetron treatment and after alosetron treatment in DSS-treated

TABLE 2 Brain activation during rectal distention in DSS-treated macaques

macaques (post-DSS > alosetron) showed regional brain activation at 20 ml and 30 ml rectal distention (Table 4).

3.4 | Intra-rectal pressure in naïve, untreated and DSS-treated macaques

Both naïve, untreated macaques and DSS-treated macaques demonstrated increased mean intra-rectal pressure with increased balloon volume (Figure 6). Peak intra-rectal pressure was observed at 5 s during balloon inflation.

Within the naïve, untreated macaques, intra-rectal pressure tended to remain above 0 mmHg following the start of 20 and 30 ml rectal distention for up to 30 s ($p < 0.05$ vs. time 0). Also, intra-rectal pressure was higher than 0 mmHg for up to 20 s following the start of 10 ml rectal distention ($p < 0.05$ vs. time 0). In DSS-treated macaques, however, intra-rectal pressure remained higher than 0 mmHg at all rectal distention volumes up to 30 s after the start of rectal distention ($p < 0.05$ vs. time 0).

Alosetron, administered 1 h prior to RD, significantly reduced peak intra-rectal pressure, at 20 ml and 30 ml balloon volumes, compared to that of DSS-treated macaques before alosetron administration ($p < 0.05$; Figure 7A). No significant effect of alosetron administration was observed on peak intra-rectal pressure at 10 ml rectal distention ($p > 0.05$). The intra-rectal pressure 10 to 30 s after the

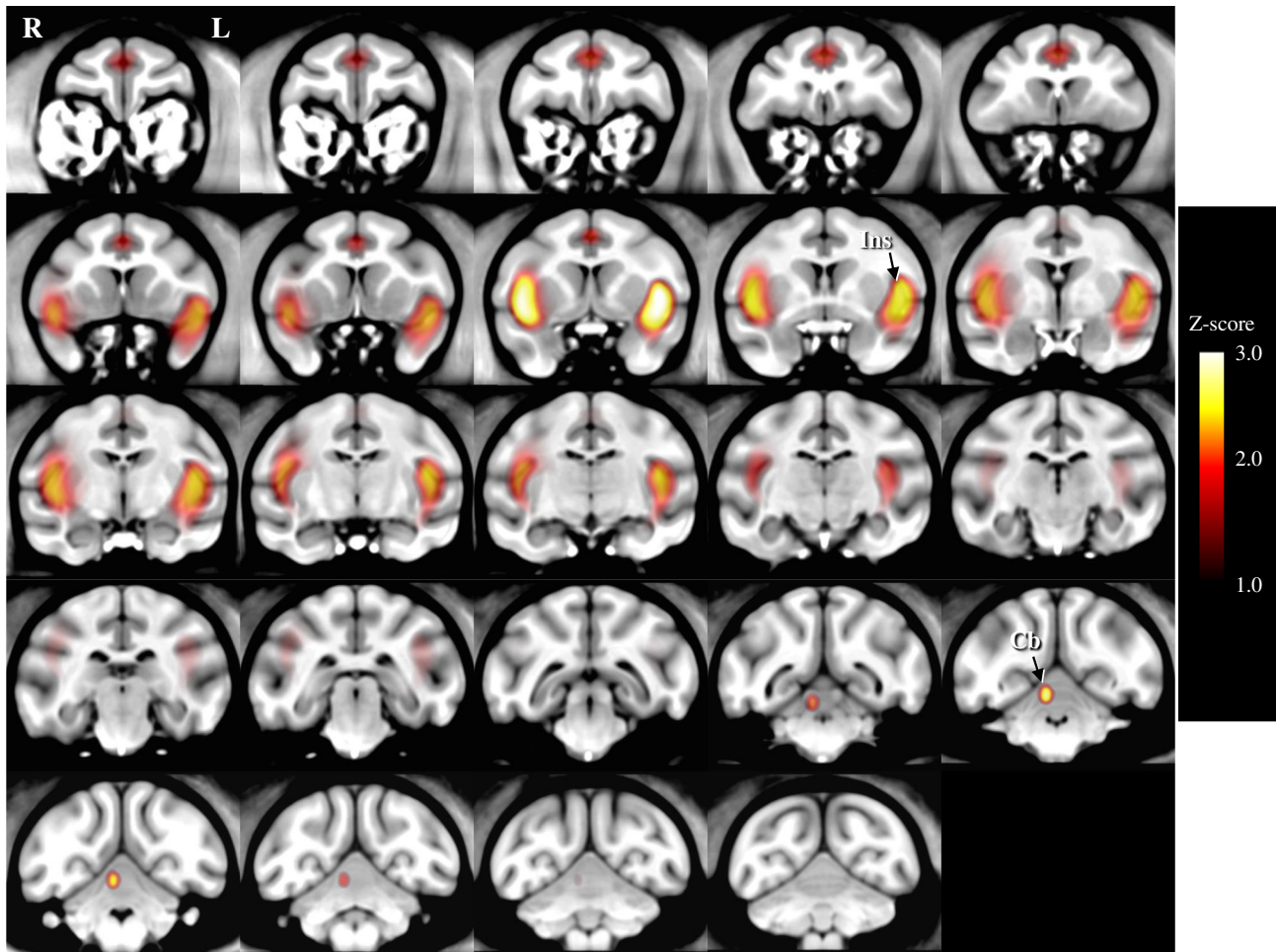


FIGURE 4 Coronal brain sections showing mean peak voxels during 30 ml rectal distention in DSS-treated macaques ($n = 4$). Significant bilateral activation of insula cortex (Ins) and cerebellum (Cb) was also observed during 20 ml rectal distention (See Table 2). Serial sections from rostral (upper left corner) to caudal (bottom right corner). R, macaque's right. L, macaque's left.

beginning of rectal distension was not significantly different before and after alosetron administration ($p > 0.05$ vs. before alosetron administration). Peak intra-rectal pressure was plotted as a function of rectal distention volume (Figure 7B). Significantly decreased peak intra-rectal pressures were observed in alosetron-administered DSS-treated macaques at 20 ml and 30 ml rectal distention (Figure 7B; $p < 0.05$ vs. post-DSS) and not at 10 ml rectal distention.

4 | DISCUSSION

Significant bilateral activation of Ins/SII and cerebellum in DSS-treated macaques was observed at rectal distention volumes that did not evoke regional brain activation in naïve, untreated macaques, suggesting the development of visceral hypersensitivity. The visceral hypersensitivity was not accompanied by visible signs of inflammation and rectal compliance was not significantly altered by previous repeated treatment of DSS. Alosetron, an agent known

to reduce visceral pain associated with IBS, significantly reduced peak intra-rectal pressure and rectal distention-evoked Ins/SII and cerebellum activation in DSS-treated macaques.

One of the barriers to successful translation from pre-clinical findings to clinically useful analgesics for IBS is the lack of a biomarker, a quantitative indicator of visceral hypersensitivity associated with IBS which could be used to discriminate potential treatments for efficacy based on pathophysiology.^{1,5} Findings from a variety of chronic pain states, including IBS, have suggested activation of common brain regions. Such regions include the “lateral pain system,” which is comprised of Ins and SII, generally involved with the sensory-discriminatory aspect of pain, and the “medial pain system,” which is comprised of limbic structures, such as the anterior CC, anterior Ins and prefrontal cortex, generally involved in the affective-emotional qualities of pain.^{16,43,44} At the same time, there are a number of differences in regional brain activation between chronic pain states, which could serve to differentiate pain states,

Region/Volume	Hemisphere	Z score	Coordinates (mm)		
			x	y	z
<i>10 ml</i>					
Insula	Left	1.09	-16	20	2
	Right	1.13	14	18	2
Cingulate cortex		1.16	0	-2	14
Cerebellum	Right	1.09	-4	-8	-10
<i>20 ml</i>					
Insula	Left	1.46	-16	20	2
	Right	1.48	14	18	2
Cingulate cortex		1.29	0	-2	12
Cerebellum	Right	1.19	-4	-8	-10
<i>30 ml</i>					
Insula	Left	1.98	-16	20	2
	Right	1.94	14	18	2
Cingulate cortex		1.47	0	-2	12
Cerebellum	Right	1.30	-4	-8	-10

Note: Group mean peak voxels of DSS-treated macaques approximately 1 h following p.o. alosetron administration during 10, 20 and 30 ml rectal distention. Mean calculated from four macaques. No significant peak voxels were observed during rectal distention following alosetron administration except the left insula during 30 ml rectal distention in DSS-treated macaques. Stereotaxic coordinates (x, y, x) are according to Horsley-Clarke's stereotaxic coordinates. Mean z score greater than 1.96, $p < 0.05$.

TABLE 3 Lack of activation following alosetron treatment during rectal distention in DSS-treated macaques

particularly those thought to be of cutaneous or visceral origin. For example, different brain region activation and the degree of activation, despite similarity in intensity of the acute painful stimulus, have been observed upon visceral and cutaneous stimulation.⁴⁵ Thus, evoked brain activation in IBS patients has potential as a tool for mechanism elucidation and could further be utilized to test therapeutics with novel mechanisms of action.¹⁶

Cerebral blood flow is utilized as an indirect indicator of regional brain functioning in both normal and pathological states. One method of quantifying cerebral blood flow is thorough ¹⁵O-water positron emission tomography (PET). Significant changes in cerebral blood flow, and thus regional functioning, have been observed in pain-related brain regions during colorectal distention in IBS patients.^{46,47} Both increased and decreased regional brain activation have been reported, which may be, in part, due to the heterogeneity of IBS patients selected for these studies and to the use of various stimulation protocols.⁴⁸ In both healthy humans and IBS patients, "intense" (40 mm Hg) colorectal distention for 80s led to bilateral activation of Ins and thalamus.⁴⁷ The CC was activated only in healthy humans. Tanaka et al. did not indicate if "mild" (20 mm Hg) colorectal distention activated brain regions in either IBS patients or healthy controls and also did not indicate whether subjective pain rating between healthy controls and IBS patients differed at either "mild" or "intense" colorectal distention.⁴⁷ Thus, whether visceral

hypersensitivity was due to a central sensitized state, of enhanced CNS responding to stimuli that was previously innocuous, in these patients is not known.¹

The effects of therapeutics on both rectal distention-evoked brain activation and self-reported effects on symptoms have also been examined using PET in IBS patients.⁴⁸ In IBS patients treated with alosetron, decreased activation was observed in limbic brain regions.⁴⁹ Alosetron treatment also decreased self-rated pain evoked by recto-sigmoidal distention, thus suggesting an association between limbic area activation and pain associated with IBS.

Disadvantages of PET imaging include the requirement for a cyclotron near the imaging site to produce radio-tracers and the need to perform arterial cannulation for blood sampling to measure levels of radio-tracer.⁵⁰ As an alternative, brain functioning can be observed noninvasively and without radio-chemicals with blood oxygen level-dependent (BOLD) fMRI, wherein changes in neural tissue metabolism are related to hemodynamic changes detected by magnetic resonance of hemoglobin.

Using fMRI, 120 ml rectal distention in IBS patients evoked greater activation of Ins, prefrontal cortex and thalamus—structures involved with pain sensory-discrimination—compared to that of healthy controls.¹⁸ Self-reported pain, as assessed by visual analog scale, at a given rectal distention volume was also greater in IBS patients compared to healthy controls. In IBS patients, 90 ml rectal distention was reported to as "mild-moderate pain" and 120 ml rectal distention

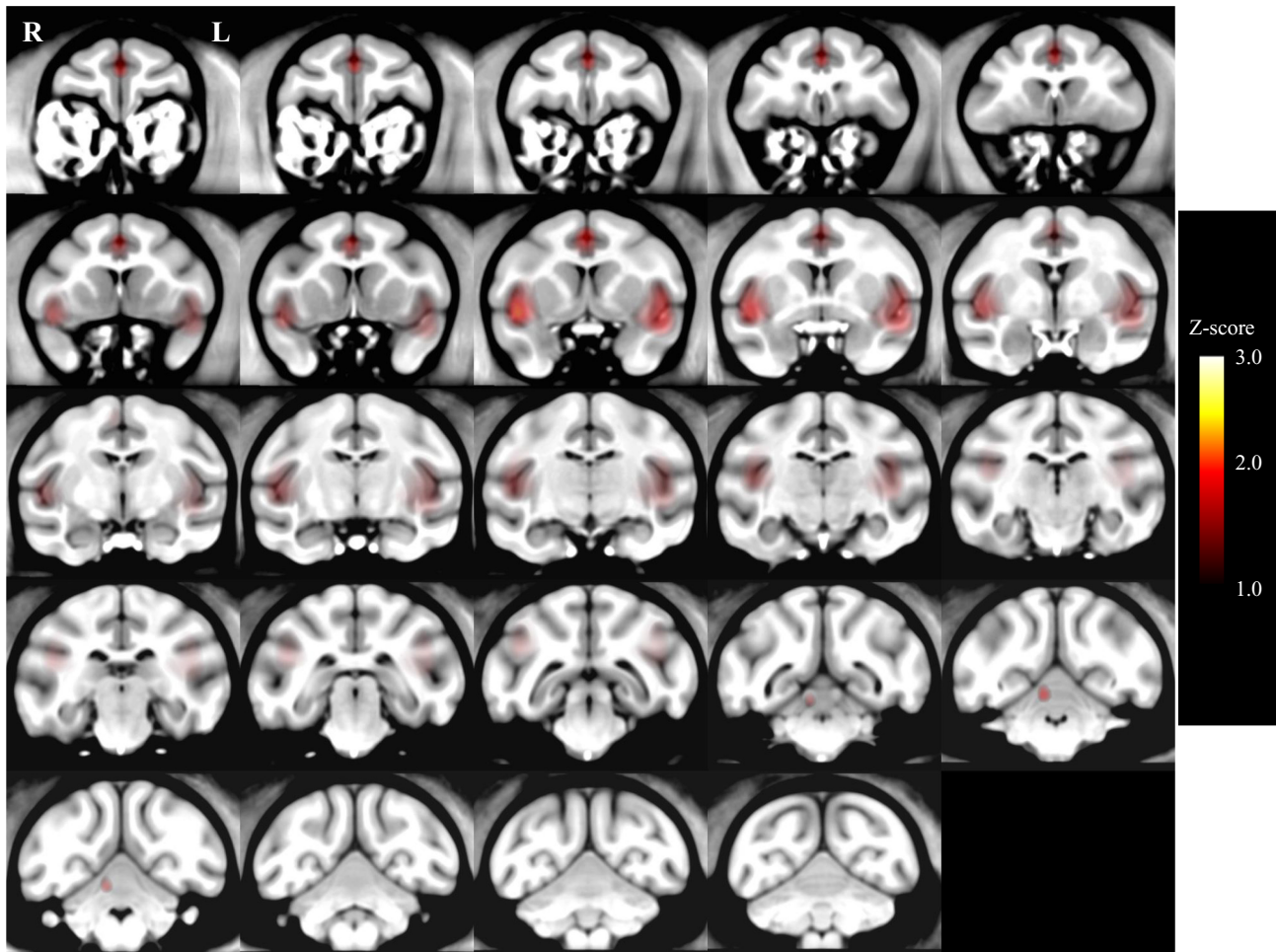


FIGURE 5 Coronal brain sections showing mean peak voxels during 30 ml rectal distention in alosetron-administered DSS-treated macaques ($n = 4$). At 30 ml rectal distention, activation of the left insula persisted (See Table 3). Serial sections from rostral (upper left corner) to caudal (bottom right corner). R, macaque's right. L, macaque's left.

was reported as “moderate–severe pain”.¹⁸ Yuan et al. did not report whether a lower rectal distention volume evoked brain activation in either IBS patients or healthy controls.¹⁹ Nonetheless, Yuan et al. demonstrated potential central sensitization and association between activation of the “lateral pain system” and pain associated with IBS. Also utilizing fMRI, Wang et al. reported activation of the cerebellum at 40, 80 and 120 ml rectal distention, in addition to Ins and thalamus in IBS patients.¹⁹ The cerebellum appears to be involved in sensory and emotional processing in addition to well known functions of motor coordination and learning.⁵¹

The current findings in nonhuman primates parallel previous findings utilizing neuroimaging. None of the rectal distention volumes evoked brain activation in naïve, untreated macaques. By contrast, in DSS-treated macaques, bilateral Ins/SII activation was observed at 20 and 30 ml rectal distention volumes. The current findings suggest similar regions of activation between DSS-treated macaques and IBS patients.

One caveat that should be addressed is that macaques in the current study were under propofol anesthesia

during brain imaging. Anesthetic doses of propofol reduce cerebral metabolism and cerebral blood flow, which could affect interpretation of data based on blood flow and oxygenation level.⁵² In healthy volunteers rendered unconscious with clinical doses of propofol, processing of intense noxious stimulation between the spinal cord and cortical regions, visualized with fMRI, was not altered, but responses to both innocuous and moderately noxious stimulation were diminished.⁵³ In a previous study in naïve macaques, significant activation of contralateral Ins/SII during noxious cutaneous pressure stimulation of the foot was observed during infusion of anesthetic doses of propofol.⁴⁰ In the current study, in naïve macaques, no significant brain activation was observed up to 30 ml rectal distention and this could have been in part due to propofol anesthesia. More likely, however, is that the rectal distention volumes used in the current study were not noxious. In DSS-treated macaques, however, 20 and 30 ml rectal distention evoked robust brain activation. Thus, central sensitization following repeated DSS treatment

Region/volume	Hemisphere	Z score	Coordinates (mm)		
			x	y	z
<i>10 ml</i>					
Insula	Left	1.39	-16	20	2
	Right	1.45	14	18	2
Cingulate cortex		1.51	0	-2	14
Cerebellum	Right	1.48	-4	-8	-10
<i>20 ml</i>					
Insula	Left	2.59	-16	20	2
	Right	2.68	14	18	2
Cingulate cortex		2.01	0	-2	12
Cerebellum	Right	2.39	-4	-8	-10
<i>30 ml</i>					
Insula	Left	2.81	-16	20	2
	Right	3.01	14	18	2
Cingulate cortex		1.98	0	-2	12
Cerebellum	Right	2.61	-4	-8	-10

TABLE 4 Contrast mapping between post-DSS-treated and DSS-treated macaques after alosetron administration during rectal distention

Note: Group mean contrast maps of post-DSS-treated macaques and DSS-treated macaques approximately 1 h following p.o. alosetron administration during 10, 20 and 30 mL rectal distention. (post-DSS > post-DSS alosetron). Mean contrast calculated from four macaques. Significant reductions in distention-evoked activation of bilateral insula and right cerebellum following alosetron treatment compared with post-DSS treated macaques. Stereotaxic coordinates (x, y, x) are according to Horsley-Clarke's stereotaxic coordinates. Mean z score greater than 1.96, $p < 0.05$. z score greater than 2.58, $p < 0.01$.

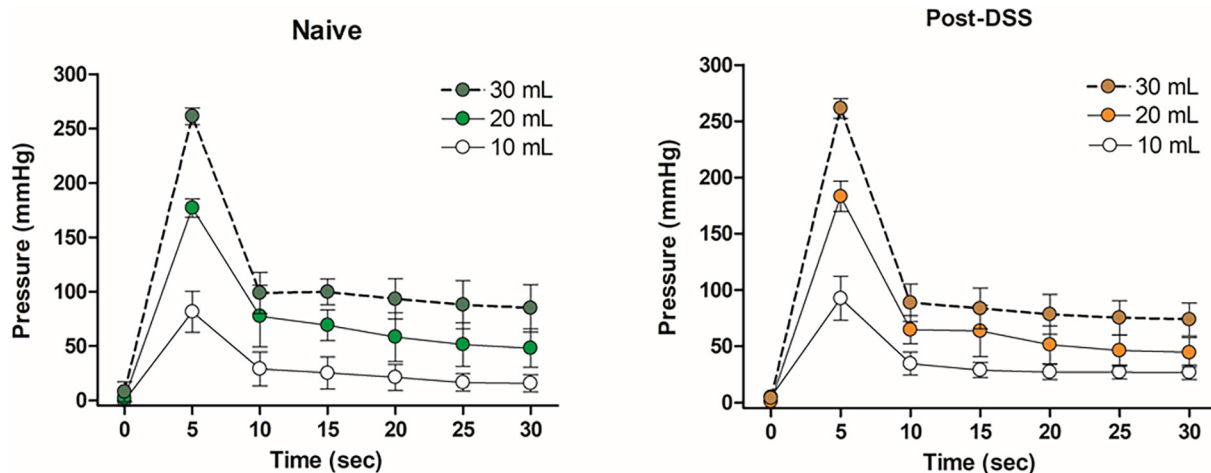


FIGURE 6 Rectal compliance following rectal balloon distention in naïve, untreated macaques and DSS-treated macaques (“post-DSS”). Peak intra-rectal pressure was observed at 5 s of inflation. Increasing balloon volumes increased intra-rectal pressure. There were no statistically significant differences between naïve and post-DSS macaques in intra-rectal pressure at each balloon volume at each time point. Data shown are mean (\pm SD). Naïve, $n = 4$. DSS-treated, $n = 4$.

can be visualized despite systemic propofol anesthesia. Treatments that reduce visceral hypersensitivity could be visualized as reduced regional brain activation.¹⁶

Following alosetron treatment, decreased activation of the “emotional motor system,” including the amygdala and hypothalamus, was observed in IBS patients using ¹⁵O-water

PET.⁴⁹ Alosetron treatment in IBS patients reduced rectal distention-evoked activation of the orbitofrontal cortex and medial temporal gyrus in IBS patients.^{28,49} Jarcho et al. noted that IBS patients who responded best to alosetron treatment were those who had lower levels of baseline, pre-treatment rectal distention-evoked orbitofrontal cortex activation.²⁸

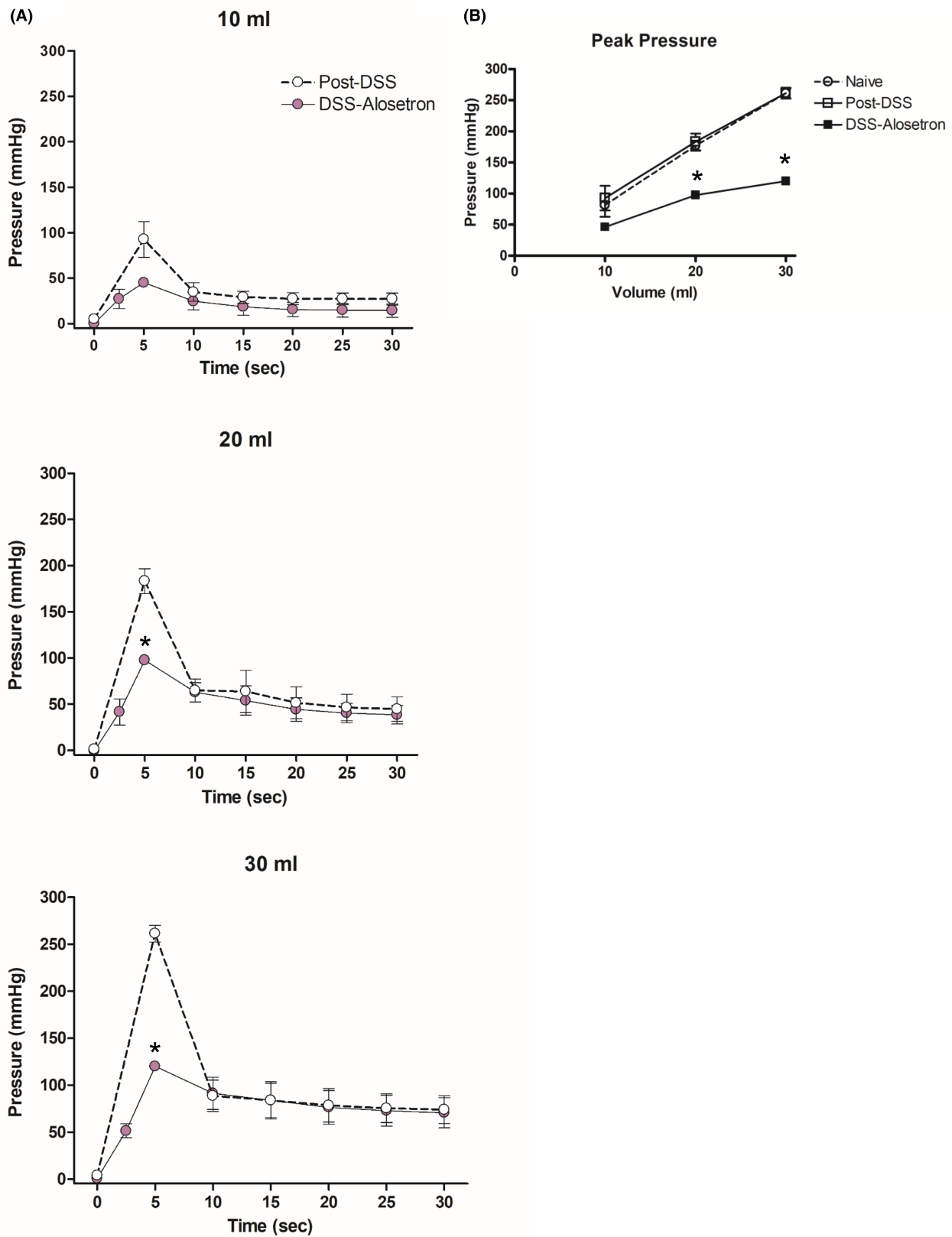


FIGURE 7 Effect over time of alosetron administration during rectal distention DSS-treated macaques (“post-DSS”). (A) Alosetron administration (“DSS-Alosetron”) 1 h p.o. prior to testing significantly reduced peak intra-rectal pressure, at 5 s, at 20 and 30 ml rectal volumes but not at 10 ml. (B) Peak intra-rectal pressure as a function of balloon volume. Naive, untreated, $n = 4$. Post-DSS and DSS-Alosetron, $n = 4$. Data expressed as Mean (\pm SD). $p < 0.05$ vs. post-DSS.

This result suggests that sensitivity to evoked brain activation could be used to predict patient responses to treatment and to guide individual treatment.

However, no significant changes in brain regions associated with “intensity of pain,” such as the Ins, were observed despite a significant decrease in self-reported pain scores.⁴⁹ The PET findings suggest that reduced pain perception following alosetron treatment could be related to decreased IBS-associated anxiety, as suggested by reduced activation of the “emotional motor system”. The effect of alosetron on the “lateral pain system” in IBS patients has yet to be reported.

The current findings in DSS-treated macaques indicate that the clinical analgesic effect of alosetron is also due to inactivation of the “lateral pain system”. Inactivation of Ins in other clinical pain states and in nonhuman primate pain models is associated with decreased pain hypersensitivity—treatments that do not reduce Ins activation are not significantly antinociceptive.^{54–56} Testing other clinically used IBS therapeutics will be needed to confirm congruence between clinical pain relief and regional brain inactivation and to further support the notion that the Ins is a key brain region related to visceral pain and hypersensitivity associated with IBS.

One other potential barrier to preclinical development of new analgesics for IBS is current reliance on a nonhuman animal species that is neuroanatomically and phylogenetically distinct from humans.⁵ Nonhuman primates could be utilized as a preclinical model species not only because their neuroanatomy and genetics are closer to humans than rodents but standard clinical imaging equipment can be utilized. In the current study, a 3.0 T MRI was used. However, for small animals such as rodents, a small-bore animal MRI with high magnetic field is required. Also, while there are similarities, for example, at the genomic level between some mouse and human peripheral tissues such as small intestine and liver,⁵⁷ there are significant differences between mouse and human dorsal root ganglion and CNS neurons, indicating species-specific functioning at the cellular and organ levels.⁵⁷ In addition, potential therapeutics (for inflammatory bowel disease) tested in rodent models have not consistently demonstrated clinical efficacy, which implies fundamental differences in processes between the mouse disease model and the clinical disease state.^{11–14} Thus, caution must be exercised in translating directly from mouse models to clinical IBS. Mechanistic understanding and evaluation of novel therapeutics in a second species, such as nonhuman primates, could lower the barrier to successful clinical translation.⁵⁸

One limitation of the current study is that the exact neural mechanism mediating Ins and cerebellar activation during rectal distention in the DSS-treated macaque and inactivation following alosetron treatment can only be speculated. Primary afferent neurons convey nociceptive information

to the spinal cord dorsal horn and synapse with ascending spinothalamic neurons terminating in the ventroposterior thalamus.⁵⁹ Spinothalamic neurons then synapse with thalamic neurons that project to the “lateral pain system”. Thus, decreased Ins activation following alosetron treatment could have occurred due to inactivation of presynaptic structures enriched with 5-HT₃ receptors, including the gut, peripheral and CNS neurons or due to disinhibition of yet to be identified inhibitory interneurons.^{29,60} At the same time, it is possible that peripheral tissues, including gut, peripheral visceral, sensory and autonomic nerves could have also been sensitized, which in turn led to initiating central sensitization or maintaining this state. Further examination of the role of peripheral tissues are needed to confirm their potential role.

AUTHOR CONTRIBUTIONS

Study design and concept by R. Fujii, T. Natsume and H. Takamatsu. Data collection by R. Fujii, Y. Awaga, K. Nozawa and M. Matsushita and T. Natsume. Data analysis by R. Fujii, K. Nozawa and M. Matsushita and T. Natsume, A. Hama and H. Takamatsu. Manuscript written and edited by R. Fujii, A. Hama and T. Natsume. All authors reviewed and approved the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data sets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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