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Castex, Nathalie, Jean Fioramonti, Jacques Ducos de Lahitte, Gerard Luffau, Jean More, and Lionel Bueno. Brain Fos expression and intestinal motor alterations during nematode-induced inflammation in the rat. *Am. J. Physiol.* 274 (*Gastrointest. Liver Physiol.* 37): G210–G216, 1998.—Brain-gut interactions and intestinal motility were studied during pulmonary and jejunal inflammation induced by *Nippostrongylus brasiliensis*. Jejunal electromyographic activity was continuously recorded from *day 1* before to *day 28* after infection. Expression of c-*fos* was assessed in the brain by immunohistochemistry, and myeloperoxidase (MPO) activity was determined in lung and intestine on *days 1*, *7*, *14*, *21*, and *28* postinfection. The cyclic intestinal motor pattern was replaced by an irregular activity from *day 4*, corresponding to larvae migration to the intestine, to *day 14*. c-*fos* was expressed in the caudal nucleus of the solitary tract (NTS) and lateral parabrachial nucleus (LPB) on *day 1* (lung stage of *N. brasiliensis*) and in the medial part of the NTS, the LPB, and locus ceruleus on *day 7*. Pulmonary and intestinal MPO activity was increased from *days 1* to *21* postinfection. During *N. brasiliensis* infection, c-*fos* expression indicates that specific and different brain nuclei are activated at the onset of pulmonary and intestinal inflammation, which is associated with motor disorders.

jejunal motility; *Nippostrongylus brasiliensis*-induced inflammation; brain-gut interaction

NIPPOSTRONGYLUS BRASILIENSIS infection in rats is widely used as a model for studying pathological conditions of the intestine. The life cycle of *N. brasiliensis* involves an external, free-living phase, an extraintestinal somatic migration (from skin to lungs), and a parasitic intestinal phase. The lung and enteric phases of the primary infection, which do not appear at the same time in the course of infection, produce an acute inflammatory reaction in the respective tissues (27). *N. brasiliensis* causes changes in intestinal morphology and function. Histologically, villus atrophy, crypt hyperplasia, accumulation of inflammatory cells such as leukocytes in the lamina propria, and hypertrophy of the muscularis externa can be seen (26). In infected rats, gastric emptying is unaffected (25) but the absorptive function of the jejunal mucosa is impaired. Alterations in intestinal motility have been documented during the primary infection, but only in vitro. Moreover, these results are contradictory and seem to depend on the origin of the jejunal smooth muscle, i.e., either circular or longitudinal (8). Therefore, it is difficult to anticipate what type of alteration would occur in vivo. In our study, changes in the intestinal motor profile have been monitored periodically in conscious animals over 28 days postinfection.

Changes in intestinal motility associated with inflammation were first reported many years ago and are well documented (15). They are possibly due to the effect of the inflammatory process on smooth muscle cells (17) or intrinsic nerves of the gut (13). It is well known that in the enteric nervous system, efferent and afferent neurons are altered during intestinal inflammation. However, the question arises as to the effects on the central sites receiving projections from the sensory neurons involved in intestinal inflammation. The immediate-early gene c-*fos*, which encodes for the nuclear phosphoprotein Fos, has been shown to be particularly suited to trace the pathways involved during sensory processing (16). Fos protein is expressed in specific brain sites that are activated by afferent stimulation occurring after visceral noxious stimuli (11) or intestinal anaphylaxis (4). Detection of Fos expression may be considered a useful tool in the determination of different brain areas that may be activated during pulmonary and intestinal inflammation. Moreover, the benefit of the *N. brasiliensis* model is that during its life cycle the parasite induces both pulmonary and intestinal inflammation at two different times (1 and 7 days, respectively). Consequently, we proposed to explore the effects of such inflammation on supraspinal sites and to attempt to correlate the sites in the central nervous system (CNS) with the sites of inflammation.

In our study, intestinal and pulmonary inflammation have been assessed by the activity of myeloperoxidase (MPO), an enzyme found in high concentrations in myeloid-derived cells that reflects the degree of polymorphonuclear leukocyte infiltration (21).

Consequently, using recordings of intestinal myoelectric activity, determination of MPO in the lungs and intestine, and detection of Fos expression in some brain nuclei, the present study was designed to analyze the temporal and spatial relationships between small intestinal motility changes, inflammation, and brain information associated with the course of *N. brasiliensis* infection. These parameters were determined on *days 1*, *7*, *14*, and *28* postinfection, which were selected according to specific time points of infection development.

MATERIALS AND METHODS

Animal Preparation

Experiments were performed in 48 male Wistar rats (Janvier, Le Genest Saint Isle, France) initially weighing between

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150 and 200 g. Two groups of six rats were used for electromyographic studies and six groups of six rats were used for histological and immunohistological analysis and MPO determination. Rats were housed under controlled lighting conditions, with standard diet (Usine d'Alimentation Rationnelle, Epinay-sur-Orge, France) and water provided ad libitum. All protocols were approved by the Local Animal Care and Use Committee of Institut National de la Recherche Agronomique.

Culture and Infection Techniques

N. brasiliensis was maintained in continuous culture by harvesting worm eggs from infected rats and growing them to the third larval stage on vermiculite and filter paper, using a technique described by Jennings et al. (14). The appropriate rats were infected by subcutaneously injecting 6,000 third-stage infective larvae of *N. brasiliensis* in 0.5 ml sterile saline into the rat flank. Controls received sterile saline only.

Intestinal Myoelectric Studies

The animals were prepared for long-term recordings of intestinal myoelectric activity with the use of a previously described technique (23). Briefly, under ketamine anesthesia (120 mg/kg ip; Imalgene 1000, Rhône Merieux, Lyon, France), two groups of three insulated Nichrome wire electrodes (80-μm diameter, 80-cm length) were implanted in the wall of the proximal jejunum 5 and 15 cm distal to the ligament of Treitz. These sites were chosen according to the observation that *N. brasiliensis* is primarily implanted in the proximal half of the small intestine (7). Electrodes were exteriorized on the back of the neck and protected by a glass tube attached to the skin. Electromyographic recordings were started 3 days after surgery.

Jejunal myoelectric activity was recorded with an electroencephalograph (Minihuit Alvar, Paris, France), using a paper speed of 3.6 cm/min and a time constant of 0.03 s. Spiking activity collected by bipolar derivation was summed every 20 s by an integrator circuit and automatically plotted on the *y*-axis of a potentiometric recorder (L 6514, Linseis, Selb, Germany) with a paper speed of 6 cm/h. This integrated record permitted a clear determination of the cyclic occurrence of migrating myoelectric complexes (MMC). Animals were deprived of food from 8 AM to 5 PM to obtain a fasted pattern of motility characterized by recurring MMC. Motility was appraised by counting the number of MMC each day from 11 AM to 5 PM. At the end of MMC recording (5 PM), 12 g of rat chow was given to the animals.

Evaluation of Inflammation

Under urethan anesthesia (1 g/kg ip), just before intracardiac perfusion for Fos immunohistochemistry, samples of lung and intestine were taken for MPO activity measurement and histological control.

MPO activity measurement. MPO activity was determined with the use of the slightly modified method of Bradley et al. (1). Segments (200–250 mg) of intestine (8–10 mm in length, taken at 15 cm distal to the ligament of Treitz) or lung were finely minced at 4°C and homogenized with a Polytron for 30 s in 10 mM potassium phosphate buffer (pH 6.0) with 0.5% hexadecyltrimethylammonium bromide in an approximate ratio of 50 mg of tissue per 1 ml. This suspension was sonicated for 30 s, and an aliquot (1.5 ml) was then frozen and thawed twice for the full release of MPO stored in the azurophilic granules of polymorphonuclear neutrophils. After centrifugation at 15,000 *g* for 15 min at 4°C, an extract (5–100 μl) of the final supernatant was mixed with 3 ml of 10 mM potassium phosphate buffer (pH 6.0) containing 0.07% of 20 mM aqueous guaiacol and 20 μl of 3% hydrogen peroxide. Absorbance at 470 nm was determined with a spectrophotometer (Uvikon 860, Kontron Instruments, Basel, Switzerland) for 2 min. One unit of MPO activity was defined as the quantity catalyzing the decomposition of 1 μmol of hydrogen peroxide to water per minute at 37°C. Protein content was measured by absorbance with a Bio-Rad protein assay kit (Bio-Rad, Ivry sur Seine, France). MPO activity was expressed in units per 100 mg of protein.

Histological control. Two pieces (5 mm) of jejunum 15 cm distal to the ligament of Treitz were fixed in Bouin's solution and Carnoy's solution, respectively. The samples were cleared in xylene and embedded in paraffin blocks, and 3-μm sections were made with a rotating microtome. Bouin's fixative was used for routine histological analysis with hematoxylin and eosin staining, whereas Carnoy's fixative was used for identification of intestinal mast cells with Alcian blue-Safranin O staining (22).

Fos Immunohistochemistry

After urethan anesthesia (1 g/kg ip), the animals were immediately perfused intracardially with 300 ml of 0.1 M phosphate-buffered saline (PBS) followed by 500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Brains were removed, postfixed for 4 h in the same fixative, and cryoprotected overnight in 30% sucrose in PB. Entire brains were cryostat cut into 40-μm coronal sections and collected in PB. Every third section was stained for c-*fos* immunohistochemistry. Free-floating sections were incubated for 30 min at room temperature in a blocking solution of 3% normal goat serum in PBS with 0.3% Triton X-100 (NGST). Sections were then incubated overnight at 4°C in the primary antiserum directed against the Fos protein (c-*fos* Ab-2; Oncogene Science, Uniondale, NY). The Fos antibody was used at a dilution of 1:2,000 in 1% NGST. The incubated sections were washed twice in 1% NGST and incubated in biotinylated goat anti-rabbit immunoglobulin G for 1 h at room temperature, then washed twice in 1% NGST and incubated for 1 h in elite avidin-biotin complex (Vectastain kit; Vector Laboratories, Burlingame, CA), according to the avidin-biotin-peroxidase method of Hsu et al. (12). Peroxidase activity was revealed using diaminobenzidine [0.05% in tris(hydroxymethyl)aminomethane buffer containing 0.2% hydrogen peroxide] as chromogene. Sections were then mounted on gelatin-coated slides, dehydrated, cleared in toluene, and coverslipped. The presence of c-*fos* immunoreactivity was detected as a dark brown reaction product in cell nuclei under a light microscope.

Brain regions were identified according to the atlas of Paxinos and Watson (20). All individual nuclear staining cells were estimated using an image-grabbing program (Neotech, Paris, France) and an Optilab image analysis software package (Graftek, Paris, France) running on an Apple Macintosh IICi computer. The average density and area of a Fos-positive cell nucleus was determined, and the grain-counting program was used to discriminate target cells from background. Only cells that had significant levels of diaminobenzidine reaction product in their nucleus above tissue background levels were counted. The number of neurons exhibiting Fos were counted per side on three sections from each individual nucleus and for each animal. The total number of positive cells for each nucleus, at a defined bregma location, was then divided by the number of sections, to provide a mean cell count per side of section.

Experimental Procedure

Two groups of six rats were surgically implanted with chronic electrodes 4 days before either injection of *N. brasiliensis* larvae or vehicle (0.9% NaCl). Recordings of jejunal

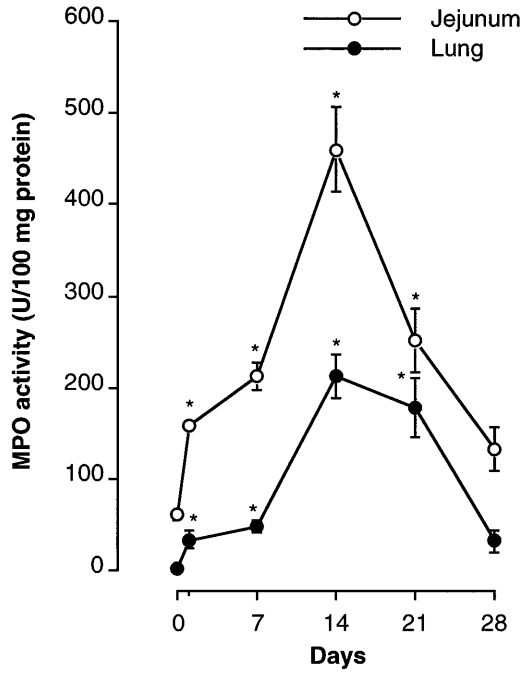

Fig. 1. Intestinal and lung myeloperoxidase (MPO) activity over time course of *N. brasiliensis* infection in rats (means ± SE; $n = 6$). *Significantly ($P < 0.05$) different compared with uninfected control.

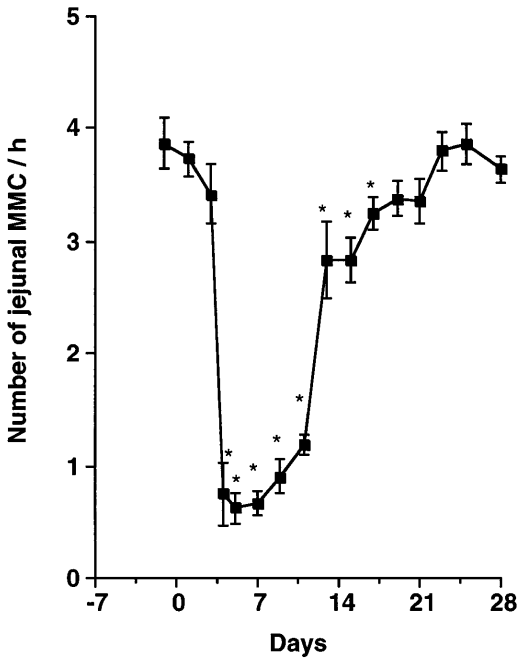

Fig. 3. Number of MMC per hour during *N. brasiliensis*-induced inflammation in rats (means ± SE; $n = 6$). *Significantly ($P < 0.05$) different from control values.

electromyograms began 1 day before injection (parasite or saline) and lasted until 28 days postinfection. The myoelectric activity was monitored periodically over 28 days postinfection of *N. brasiliensis* or vehicle.

Five other groups of six rats were also infected by *N. brasiliensis* and killed between 2:00 PM and 3:30 PM at five different time points over the course of infection. Determination of MPO activity, histology, and brain Fos detection were performed on *days 1*, *7*, *14*, *21*, and *28* postinfection, as described above. A last group was used as control to determine an eventual effect of subcutaneous injection. The rats in this group received vehicle and were killed 24 h later for histological control, determination of MPO activity, and Fos immunohistochemistry.

Statistical Analysis

Results are expressed as means ± SE. Comparisons were performed using analysis of variance and Student's *t*-test for unpaired values. Statistical significance was accepted if $P < 0.05$.

RESULTS

Evaluation of Inflammation

Histological control. Histological changes similar to those previously reported were observed during *N. brasiliensis* infection (26, 27).

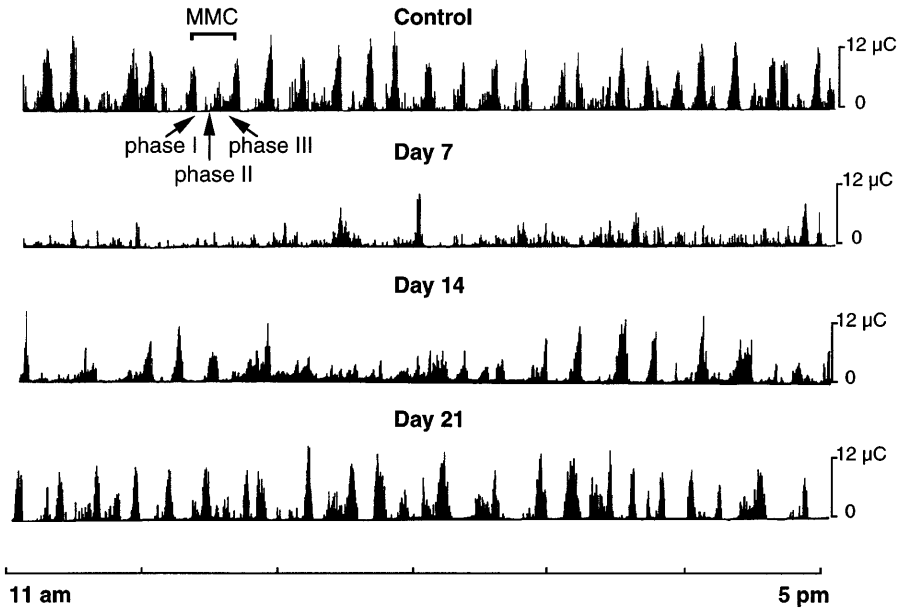

Fig. 2. Integrated records of myoelectric activity of proximal jejunum (5 cm distal to ligament of Treitz) before and after *N. brasiliensis* infection. Note large inhibition of migrating myoelectric complexes (MMC) occurring on *day 7* (µC: microcoulombs).

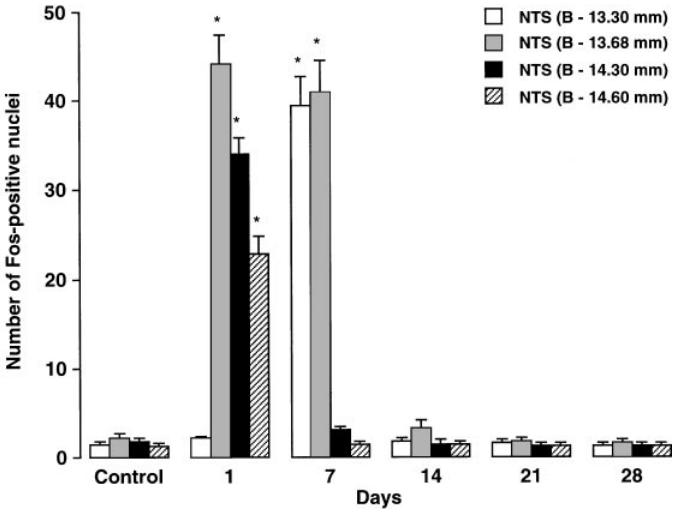

Fig. 4. Quantitative rostrocaudal distribution of Fos-positive cells in nucleus of the solitary tract (NTS) evoked by *N. brasiliensis* infection. Bars represent means of Fos-positive cells counted on one side of a section for 3 defined sites of NTS (means ± SE; $n = 6$). *Significantly ($P < 0.05$) different from control values. B, bregma.

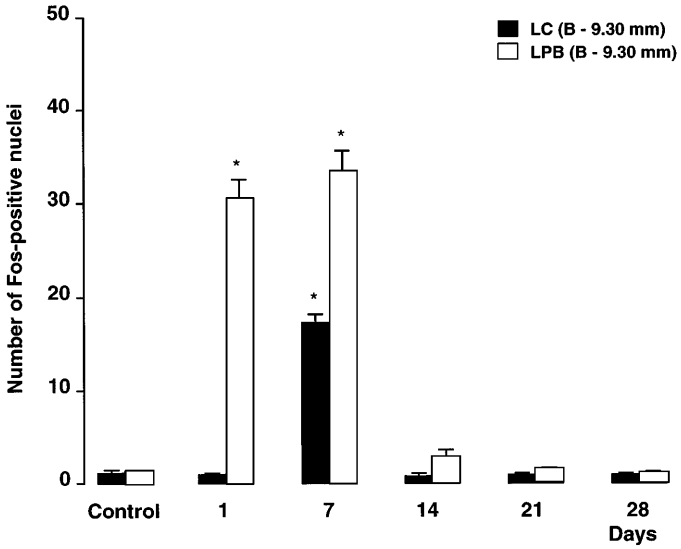

Fig. 6. Number of Fos-positive cells in lateral parabrachial nucleus (LPB) and locus ceruleus (LC) induced by *N. brasiliensis* infection. Bars represent means of Fos-positive cells counted on one side of a section for 3 defined sites of LPB and LC (means ± SE; $n = 6$). *Significantly ($P < 0.05$) different from control values.

Briefly, on *day 1* postinfection, corresponding to the pulmonary larvae migration, lungs were characterized by hemorrhage and edema in the parenchyma. At this stage, the proximal jejunum was normal and jejunal villi were spatulated and well defined, with a slight infiltration of eosinophils in the lamina propria. On *day 7* villi were partially fused, edematous, and distended. An accumulation of inflammatory cells (neutrophils and eosinophils) was seen in the lamina propria, and numerous cells in the Lieberkühn glands were undergoing mitosis. The acute inflammatory reaction of the mucosa was less important on *days 14*, *21*, and *28*, with a slight edema but numerous foci of cellular infiltration in the lamina propria.

On *day 1* no change was observed in mast cell number in comparison with controls. At *day 7* a few stained mast cells were visualized. On *day 14* mastocytosis in the lamina propria was evident, and on *days 21* and *28* mast cells were too numerous to be counted accurately.

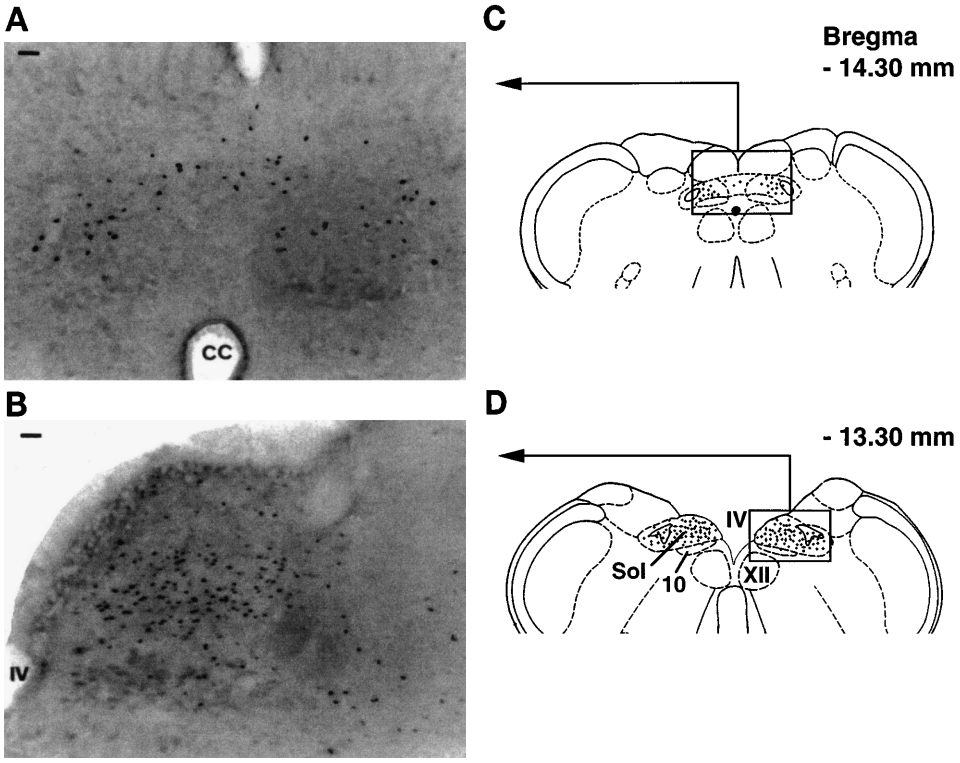

Fig. 5. Fos labeling observed on *day 1* (*A*) and *day 7* (*B*) after *N. brasiliensis* infection in NTS. *C* and *D*: diagrammatic images of location and approximate density of Fos-positive cells in NTS. Stereotaxic coordinates according to Paxinos and Watson (20) are as follows: bregma −14.30 mm (*C*) and −13.30 mm (*D*). Sol, NTS; 10, dorsal motor nucleus of the vagus nerve; XII, hypoglossal nucleus; CC, central canal; IV, fourth ventricle. Scale bar = 100 μm.

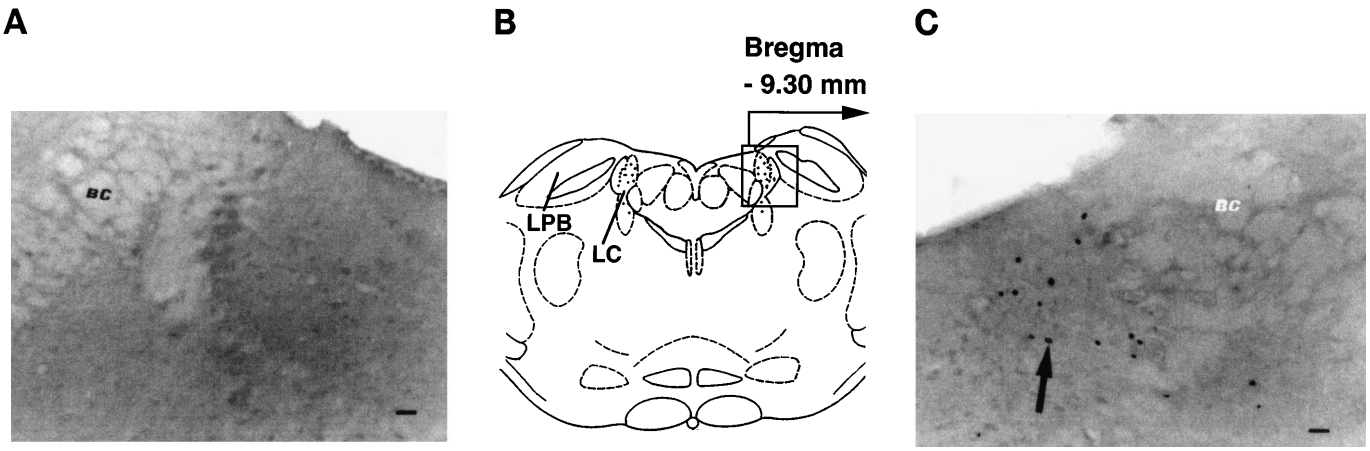

Fig. 7. Photomicrographs showing c-*fos* expression in LC from rats 7 days after *N. brasiliensis* infection (*C*) and after vehicle injection (*A*). Arrow in *C* indicates labeled neurons in LC. *B*: location and approximate density of Fos-positive cells in the LC (cartoon image). BC, brachium conjunctivum. Scale bar = 100 μm.

MPO Response

In control rats, MPO activity in the jejunum was 60 ± 5 U/100 mg protein. In *N. brasiliensis*-infected rats, MPO activity increased significantly ($P < 0.05$) on *day 1* (158 ± 4 U/100 mg protein) and *day 7* (210 ± 7 U/100 mg protein) and peaked on *day 14* (460 ± 47 U/100 mg protein). Twenty-eight days postinfection, MPO activity (132 ± 23 U/100 mg protein) was not significantly different ($P > 0.05$) from control values (Fig. 1).

As in the intestine, MPO activity in the lungs was significantly ($P < 0.05$) increased on *day 1* (33 ± 9 vs. 2 ± 0.7 U/100 mg protein) and *day 7* (44 ± 3 U/100 mg protein) and peaked on *day 14* postinfection (213 ± 23 U/100 mg protein). On *day 28*, MPO activity (31 ± 12 U/100 mg protein) did not differ significantly from control values after displaying an intermediate value on *day 21* (178 ± 32 U/100 mg protein) (Fig. 1).

Intestinal Myoelectric Activity

In the control period, corresponding to the day before *N. brasiliensis* infection, the fasted intestinal motor profile was characterized by the recurrence of MMC at a mean interval of 15.2 ± 2.4 min. Each MMC consisted of irregular activity (phase II) lasting 4–6 min, followed by a period (4–5 min) of intense and regular spiking activity (phase III). These two periods of activity were separated by a short (2–3 min) quiescent period (phase I) (Fig. 2).

Phase III, the most distinctive phase of the MMC, was used to determine the presence or absence of normal interdigestive myoelectric activity. From *day 4* to *days 13–14* postinfection, the regular occurrence of MMC was disrupted and replaced by a low level of phase II-like spiking activity with the irregular occurrence of very little phase III activity (Figs. 2 and 3). Afterwards, the motor profile was progressively restored and was normalized by *day 21*. In comparison with noninfected rats, phase III frequency was significantly reduced ($P < 0.001$) from *day 4* to *day 18* postinfection (Fig. 3).

Brain Fos Expression

Brain Fos was analyzed from three different sections of the NTS. One day after infection by *N. brasiliensis*, rat brains presented a marked c-*fos* labeling in the NTS, extending from bregma −13.68 mm to −14.60 mm at a medial location (Figs. 4 and 5*A*). Induction of c-*fos* was also observed in the lateral parabrachial nucleus (LPB) (Fig. 6), whereas no Fos was present in the locus ceruleus (LC) (Fig. 6).

On *day 7* after infection, many labeled neurons were found in the medial subdivision of the NTS from bregma −13.30 mm to bregma −13.68 mm (Figs. 4 and 5*B*). By contrast, Fos was not expressed in more caudal parts of the NTS (bregma −14.30 to −14.60 mm), as it was on *day 1* (Fig. 4). Fos-positive nuclei were identified in the LC (Fig. 7*C*) and the LPB (Fig. 6). A few labeled neurons have been seen on the commissural nucleus of the NTS, but none on the area postrema and the paraventricular nucleus at any stage of the infection. On *days 14*, *21*, and *28* no Fos expression was detected in the CNS (Figs. 4 and 6).

DISCUSSION

Our results indicate that significant changes occur in jejunal smooth muscle function 4 days after infection with *N. brasiliensis*. Leukocyte infiltration assessed by MPO activity appears in both lung and small bowel within 1 day after infection and continues for 3 wk. The present study also shows that c-*fos* expression occurs in different CNS areas according to the origin of the stimulus, i.e., pulmonary or intestinal.

Subcutaneously administered infective L3 stage larvae of *N. brasiliensis* necessarily produce a host lung stage, with associated pathology, before their arrival in the small intestine at stage L4 (27). Our study shows that the normal jejunal motor profile is disrupted and inhibited when the parasite invades the intestinal mucosa, i.e., on *day 4*. This result may be due to a

decreased responsiveness of the circular smooth muscle to both cholinergic stimulation and β-adrenergic inhibition triggered by the acute inflammation induced by *N. brasiliensis* (8). However, increased contractility of intestinal longitudinal muscle to serotonin and acetylcholine has been reported in rats 8 days after infection with the same parasite (10). These contradictory observations emphasize the fact that in vivo models involve complex mechanisms and that several processes are involved in the control of intestinal motility.

The effect of parasite infection on intestinal transit also appears to be dependent on the segment of the intestine considered, the time at which transit is studied, and the type of infection. Farmer (9), studying propulsive activity 15 min after infusion of a marker in the duodenum, found no change in the overall intestinal transit on *days 6*, *10*, *12*, and *14* after infection. However, a decrease in transit time on *day 6* and an increase on *day 8* were observed in the upper small intestine. Our study, with electrodes located in a proximal site, shows an inhibition of the jejunal motility during this period and thus provides a physiological basis for such a decreased transit time. Likewise, in the *Trichinella spiralis* inflammation model, MMC frequency and intestinal spiking activity were decreased during the 10 days studied after infection (6, 19). Worm elimination by the host occurs between *days 12* and *18* postinfection. This period corresponds, in our study, to a progressive return to a normal MMC profile and can be associated with the immune-mediated expulsion of the worm population.

The observed increase in MPO activity in the intestine and lungs of infected rats seems to be independent of the presence of the worm in the respective tissues and seems to occur as a result of the inflammatory response of the host (5). Indeed, leukocyte infiltration present in the intestine during the lung stage of the larvae suggests a signal from the parasite that modifies the final intestinal site. This hypothesis is supported by previous findings showing digestive secretory changes observed with substances released by larvae of *Haemonchus contortus* sealed in a dialysis bag placed in the stomach of lambs (3). Moreover, we find that the MPO activity peak (*day 14*) does not correspond to the maximum inhibition of intestinal motility (from *day 4* to *day 10*).

We also confirmed by histological evaluation that the number of leukocytes (28) and mast cells (27) was increased in the intestinal mucosa when the worms were eliminated after *day 14*. However, elevated MPO activity remains for several days after most worms are expelled. This suggests a continued host response to the initial injury caused by the establishment of the parasite and possibly to the injury mediated through the elicitation of immune responses, such as the Arthus reaction (21).

N. brasiliensis was used principally as an inflammatory agent in our study because this parasite provides a well-controlled stimulus for inducing injury in the lung and the small intestine. During the time course of this inflammation, remodeling of nerves occurs in the intestinal wall (24). In our work, brain c-*fos* expression further demonstrates a brain-gut interaction. In the brain stem of infected rats, we found that c-*fos* is expressed, according to the time course of the development of parasitic infection, on *days 1* and *7* and disappears on *day 14*. The distribution of c-*fos* labeling within the CNS seems to correlate with histological evidence of the onset of the acute inflammation and with the spatial location (lungs vs. intestine) of the inflammatory stimulus. The presence of c-*fos* labeling 1 day after the induction of the infection is in good agreement with the acute inflammation of the lung induced by larvae. Likewise, the increase in the number of Fos-positive neurons at 1 wk corresponds to the acute intestinal stage previously defined. However, central areas receiving neuronal input are different according to the origin of the stimulus, suggesting different afferent pathways. Indeed, inflammation occurring in lungs corresponds to a c-*fos* distribution in the NTS more caudal than when inflammation reaches the intestine. The activation of LPB neurons by the two sites of inflammation is consistent with anatomic studies showing that neurons of the NTS project to the parabrachial nucleus (18). The labeling observed in the LC during intestinal inflammation emphasizes the role of this nucleus in the response to specific digestive stimulation (2).

Finally, our results emphasize that neuronal afferents to brain are activated at the onset of pulmonary and intestinal inflammation in a specific manner. The time courses of inflammation, brain Fos expression, and changes in the intestinal motor profile suggest that motility disturbances are caused by intestinal inflammation. However, despite Fos expression in the brain we cannot determine whether motility disturbances involve the CNS or are locally mediated.

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REFERENCES

1. **Bradley, P. P., D. A. Priebat, R. D. Christensen, and G. Rothstein.** Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J. Invest. Dermatol.* 78: 206–209, 1982.
2. **Bonaz, B., and Y. Taché.** Induction of Fos immunoreactivity in the rat brain after cold-restraint induced gastric lesions and fecal excretion. *Brain Res.* 652: 56–64, 1994.
3. **Buéno, L., C. Hondé, G. Luffau, and J. Fioramonti.** Origin of the early digestive disturbances induced by *Haemonchus contortus* infection in lambs. *Am. J. Vet. Res.* 43: 1194–1199, 1982.
4. **Castex, N., J. Fioramonti, M. J. Fargeas, and L. Buéno.** c-*fos* expression in specific rat brain nuclei after intestinal anaphylaxis: involvement of 5-HT_3 receptors and vagal afferent fibers. *Brain Res.* 688: 149–160, 1995.
5. **Collins, S. M., P. Blennerhassett, and D. L. Vermillion.** Impaired acetylcholine release in the inflamed rat intestine is T

cell independent. *Am. J. Physiol.* 263 (*Gastrointest. Liver Physiol.* 26): G198–G201, 1992.
6. **Cowles, V. E., and S. K. Sarna.** *Trichinella spiralis* infection alters small bowel motor activity in the fed state. *Gastroenterology* 101: 664–669, 1991.
7. **Croll, N. A.** The location of parasites within their hosts: the influence of host feeding and diet on the dispersion of adults of *Nippostrongylus brasiliensis* in the intestine of the rat. *Int. J. Parasitol.* 6: 441–448, 1976.
8. **Crosthwaite, A. I. P., J. D. Huizinga, and J. E. T. Fox.** Jejunal circular muscle motility is decreased in nematode-infected rat. *Gastroenterology* 98: 59–65, 1990.
9. **Farmer, S. G.** Changes in the responsiveness of intestinal smooth muscle to agonists in rats infected with *Nippostrongylus brasiliensis*. *Br. J. Pharmacol.* 74: 199–205, 1981.
10. **Farmer, S. G., J. M. Brown, and D. Pollock.** Increased responsiveness of intestinal and vascular smooth muscle to agonists in rats infected with *Nippostrongylus brasiliensis*. *Arch. Int. Pharmacodyn. Ther.* 263: 217–227, 1983.
11. **Hammond, D. L., R. Presley, K. R. Gogas, and A. K. Basbaum.** Morphine or U-50,488 suppresses Fos protein-like immunoreactivity in the spinal cord and nucleus tractus solitarii evoked by a noxious visceral stimulus in the rat. *J. Comp. Neurol.* 315: 244–253, 1992.
12. **Hsu, S., L. Raine, and H. Fanger.** Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J. Histochem. Cytochem.* 29: 577–580, 1981.
13. **Jacobson, K., K. McHugh, and S. M. Collins.** Experimental colitis alters myenteric nerve function at inflamed and noninflamed sites in the rat. *Gastroenterology* 109: 718–722, 1995.
14. **Jennings, F. W., W. Mulligan, and G. M. Urquhart.** Variables in X-ray "inactivation" of *Nippostrongylus brasiliensis* larvae. *Exp. Parasitol.* 13: 367–373, 1963.
15. **Kern, F. J., T. P. Almy, F. K. Abbot, and M. D. Bogdonoff.** Motility of the distal colon in nonspecific ulcerative colitis. *Gastroenterology* 19: 492–503, 1951.
16. **Morgan, J. I., and T. Curran.** Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes *fos* and *jun*. *Annu. Rev. Neurosci.* 14: 421–455, 1991.
17. **Muller, M. J., J. D. Huizinga, and S. M. Collins.** Altered smooth muscle contraction and sodium pump activity in the inflamed rat intestine. *Am. J. Physiol.* 257 (*Gastrointest. Liver Physiol.* 20): G570–G577, 1989.
18. **Norgren, R.** Projections from the nucleus of the solitary tract in the rat. *Neuroscience* 3: 207–218, 1978.
19. **Palmer, J. M., and G. A. Castro.** Anamnestic stimulus-specific myoelectric responses associated with intestinal immunity in the rat. *Am. J. Physiol.* 250 (*Gastrointest. Liver Physiol.* 13): G266–G273, 1986.
20. **Paxinos, G., and C. Watson.** *The Rat Brain in Stereotaxic Coordinates* (2nd ed.). New York: Academic, 1986.
21. **Perry, R. H.** Transfer of immunity of *Trichinella spiralis* from mother to offspring. *J. Parasitol.* 60: 460–465, 1974.
22. **Roberts, I. S. D., C. J. P. Jones, and R. W. Stoddart.** Lectin histochemistry of the mast cell: heterogeneity of rodent and human mast cell populations. *Histochem. J.* 22: 73–80, 1990.
23. **Ruckebusch, M., and J. Fioramonti.** Electrical spiking activity and propulsion in small intestine in fed and fasted rats. *Gastroenterology* 68: 1500–1508, 1975.
24. **Stead, R. H., M. Tomioka, G. Quinonez, G. T. Simon, S. Y. Felten, and J. Bienenstock.** Intestinal mucosal mast cells in normal and nematode-infected rat intestines are in intimate contact with peptidergic nerves. *Proc. Natl. Acad. Sci. USA* 84: 2975–2979, 1987.
25. **Symons, L. E. A.** Digestion and absorption of maltose in rats infected by the nematode *Nippostrongylus brasiliensis*. *Exp. Parasitol.* 18: 12–24, 1966.
26. **Symons, L. E. A., and D. Fairbairn.** Biochemical pathology of the rat jejunum parasitized by the nematode *Nippostrongylus brasiliensis*. *Exp. Parasitol.* 13: 284–304, 1963.
27. **Taliaferro, W. H., and M. P. Sarles.** The cellular reactions in the skin, lungs and intestine of normal and immune rats after infection with *Nippostrongylus muris*. *J. Infect. Dis.* 64: 157–192, 1939.
28. **Wells, P. D.** Mast cell, eosinophil and histamine levels in *Nippostrongylus brasiliensis* infected rats. *Exp. Parasitol.* 12: 82–101, 1962.