Morphological and electrophysiological changes in mouse dorsal root ganglia after partial colonic obstruction

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Huang, Tian-Ying, and Menachem Hanani. Morphological and electrophysiological changes in mouse dorsal root ganglia after partial colonic obstruction. Am J Physiol Gastrointest Liver Physiol 289: G670–G678, 2005. First published May 26, 2005; doi:10.1152/ajpgi.00028.2005.—There is evidence that sensitization of neurons in dorsal root ganglia (DRG) may contribute to pain induced by intestinal injury. We hypothesized that obstruction-induced pain is related to changes in DRG neurons and satellite glial cells (SGCs). In this study, partial colonic obstruction was induced by ligation. The neurons projecting to the colon were traced by an injection of 1,1’-dioctadecyl-3,3,3’,3’-tetramethyldiocarbocyanine perchlorate into the colon wall. The electrophysiological properties of DRG neurons were determined using intracellular electrodes. Dye coupling was examined with an intracellular injection of Lucifer yellow (LY). Morphological changes in the colon and DRG were examined. Pain was assessed with von Frey hairs. Partial colonic obstruction caused the following changes. First, coupling between SGCs enveloping different neurons increased 18-fold when LY was injected into SGCs near neurons projecting to the colon. Second, neurons were not coupled to other neurons or SGCs. Third, the firing threshold of neurons projecting to the colon decreased by more than 40% (P < 0.01), and the resting potential was more positive by 4–6 mV (P < 0.05). Finally, the number of neurons displaying spontaneous spikes increased eightfold, and the number of neurons with subthreshold voltage oscillations increased over threefold. These changes are consistent with augmented neuronal excitability. The pain threshold to abdominal stimulation decreased by 70.2%. Inflammatory responses were found in the colon wall. We conclude that obstruction increased neuronal excitability, which is likely to be a major factor in the pain behavior observed. The augmented dye coupling between glial cells may contribute to the neuronal hyperexcitability.

dye coupling; neuronal excitability; hypertrophy; visceral pain

RESEARCH IN RECENT YEARS has provided evidence for a high degree of plasticity in the innervation of visceral organs. It was found that local injury or inflammation can alter both the intrinsic and extrinsic ganglia innervating these organs. For example, it was found that intestinal obstruction induced hypertrophy of myenteric neurons in the small intestine of experimental animals (7, 14).

An important component of the extrinsic sensory innervation of visceral organs derives from dorsal root ganglia (DRG). Whereas the physiology and pathophysiology of somatosensory innervation by DRG have been studied extensively (11), little information is available on how local injury affects DRG neurons projecting to the stomach. Similarly, inflammation in the guinea pig small intestine (23) and mouse colon (3) sensitized DRG neurons. These studies suggest that an important component of chronic visceral pain may originate at the sensory ganglia, which is in accord with studies on somatic neuropathic pain models showing that peripheral nerve injury induces ectopic firing in DRG neurons (39).

Obstruction of hollow visceral organs is a common clinical problem that may have severe consequences such as pain, sepsis, and perforation. A previous study (15) showed that obstruction of the bladder outlet caused hypertrophy of neurons in the pelvic ganglia. Information on the effect of intestinal obstruction on DRG is scarce. We are aware of only one investigation of this question, done on a model of partial obstruction of the rat small intestine (42). It was found that 3 wk after the induction of the partial obstruction, the cross-sectional area of DRG neurons innervating the obstructed region increased by 130% (42). No information is available about physiological changes in the sensory neurons during intestinal obstruction or on any changes in glial cells in these ganglia. Glial cells are considered important contributors to pain states in the central nervous system (40). We found that axotomy of the sciatic nerve in mice induced a sixfold increase in the coupling among satellite glial cells (SGCs) surrounding DRG neurons (17). This was correlated with an abnormal growth of glial processes and with a 6.5-fold increase in the number of gap junctions connecting these cells (17, 27). Similar observations were made in mouse trigeminal ganglia (9), and we hypothesized that this change might contribute to chronic pain caused by the axotomy. In the present study, we examined the effect of partial obstruction of the mouse colon on dye coupling among SGCs, on electrophysiological properties of DRG neurons, and on behavioral pain responses.

MATERIALS AND METHODS

Subjects and surgery. Experiments were done on Balb/c mice of either sex, 2–3 mo old, weighing 22–25 g. The experimental protocol was approved by the Institutional Animal Care and Use Committee. The animals were anesthetized by an intraperitoneal administration of pentobarbital sodium (48 mg/kg). All procedures were done using aseptic techniques.

To trace DRG neurons, the colon was externalized through a low abdominal midline incision onto a mat of cotton soaked with saline, and the distal colon was injected with 1,1’-dioctadecyl-3,3,3’,3’-tetramethyldiocarbocyanine perchlorate (DiI; Molecular Probes; Eugene, OR; 5% in methanol, 20 μl) (36). Eight injections with a 28-gauge needle were made circumferentially within an about 1-cm-long segment of the distal colon, 2 cm from the anus.

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To induce partial colonic obstruction, the distal colon was exteri-
ornalized as described above. A plastic tube (2 mm in diameter) was
placed along the longitudinal axis of the colon, and a silk thread was
tied around the colon and the tube. Partial obstruction was achieved
after the tube was withdrawn. The ligation was made 1.5 cm proximal
to the anus and distal to the DiI injection region. The colon was
replaced, and the incision was sutured in two layers. In sham opera-
tions, the colon was only externalized and replaced. Some control
animals were injected with DiI 8 days before the sham operation.

Tissue preparations. The animals were killed with CO₂ and DRG
L1, L4, and S1 were removed and placed in cold (4°C) Krebs solution
(pH 7.4) containing (in mM) 118 NaCl, 4.7 KCl, 14.4 NaHCO₃, 1.2
MgSO₄, 1.2 NaH₂PO₄, 2.5 CaCl₂, and 11.5 glucose. Ganglia for
intracellular recording or labeling were pinned onto the Sylgard
bottom of a chamber superfused with Krebs solution bubbled with
95% O₂-5% CO₂ at 23–24°C. For histological sections, the ganglia
and colon were fixed overnight at 4°C in 4% paraformaldehyde in
PBS (0.1 M, pH 7.4).

For mapping DRG neurons projecting to the colon, DRG L1–L6
and S1 were examined. For dye injection and intracellular recording,
we used DRG L1 and S1 from sham-operated (control) and obstructed
animals and DRG L4 from the same obstructed animals (negative
control). After identification of neurons projecting to the colon, both
dye injections and intracellular recordings were made on DRG L1 and
S1 from the sham-operated and obstructed mice.

Intracellular labeling and recording. Experiments were performed
using an upright microscope (Axioskop FS, Zeiss; Jena, Germany)
equipped with fluorescent illumination and a digital camera (Pixera
120e, Pixera) connected to a personal computer. A water-immersion
×40 objective (numerical aperture 0.8) was used in all experiments,
allowing us to visualize dye-labeled cells very clearly and to classify
them according to their morphology. Neurons and SGCs were singly
injected with the fluorescent dye Lucifer yellow (LY; Sigma Chemi-
cal; St. Louis, MO; 3% in 0.5 M LiCl solution) from a glass
microelectrode with a tip resistance of 80–120 MΩ. After identifica-
tion of neurons projecting to the colon with DiI retrograde labeling,
the SGCs near the DiI-labeled neurons were singly injected with LY.
The dye was injected by hyperpolarizing current pulses of 100 ms in
duration and 0.5 nA in amplitude at 10 Hz for 3–5 min. To determine
whether LY was injected into the DiI-labeled neurons or the SGCs
near DiI-labeled neurons, we photographed the same microscopic
field twice using the appropriate filter sets (TRITC for DiI and FITC
for LY) and then merged the two images to determine the spatial
relationship between DiI-labeled neurons and dye-injected cells. In a
series of control experiments, we found that in all cases (n = 35) when
the DiI-labeled neuron was injected with LY only the DiI-labeled
neuron was also stained with LY. As mentioned above, these obser-
vations were made with a high-power objective, which enabled
precise visual control in real time. During and after the dye injec-
tions, living neurons and SGCs were photographed. The numbers of SGCs
coupled to the dye-injected cell were counted during dye injection by
changing the microscopic focus level. This allowed us to identify the
injected cells unequivocally. Also, we found that by using this
approach rather than imaging fixed tissues, we overcame the problem
doing fading, which took place during the experiments that lasted
several hours. In some experiments, octanol (Sigma, 1.0 mM) was
added to the bathing solution. After the experiments, DRG were fixed
overnight at 4°C in 4% paraformaldehyde in PBS, washed with PBS,
and mounted in Gel/mount (Biomeda). Cells labeled with LY were
imaged with a Bio-Rad confocal microscope. For dye injection, DRG
were harvested after 4 or 6–8 days of obstruction or sham operation.

In intracellular recording, DRG were bathed in Krebs solution at
32°C. We recorded from DRG neurons that were impaled blindly and
also from the neurons projecting to the distal colon, as determined by
retrograde labeling with DiI. The microelectrodes were filled with 2 M
KCl, with tip resistances of 80–120 MΩ. Transmembrane currents
were passed through the recording electrode using the bridge circuit of
a preamplifier (model IR 283, Neuro Data Instruments). Input resis-
tance of the neurons was measured by passing hyperpolarizing cur-
cents (0.1 nA, 100 ms) and balancing the bridge. Electrophysiological
data were recorded with a video cassette recorder using a Neuro-
corder (model DR 390, Neuro Data Instruments). Neurons were
classified as displaying subthreshold oscillations when they showed
rhythmic changes in membrane potentials of at least 1 mV in ampli-
tude (1). Membrane potentials were sampled for 2 s at 2 kHz. Spectral
analysis was done with the fast Fourier transform module of PCLAMP
9 (Axon Instruments; Foster City, CA). All intracellular recording
experiments were done on DRG harvested after 6 days of obstruction
or sham operation.

Morphological studies. Two weeks after the DiI injection into the
colon wall, DRG L1–L6 and S1 were harvested. The ganglia were
pinned onto the Sylgard bottom of a dish. DiI-labeled neurons were
encoded under a microscope equipped with fluorescent illumination
using filters for TRITC. Six days after obstruction or sham operation,
DRG S1 and the colonic tissues, which were 1.5–3.5 cm proximal to
the anus, were harvested and fixed overnight at 4°C in 4% parafor-
maldehyde in PBS. The DRG and colon were then embedded in
paraffin using conventional histological techniques. Serial sections, 5
µm thick, were cut, deparaffinized in xylene, rehydrated in a graded
series of ethanol, and stained with hematoxylin-cosin. DRG sections
were imaged at ×400. The cross-sectional areas of all nucleated
neurons encountered in the field were measured using Image-Pro Plus
software (Media Cybernetics; Silver Spring, MD) and divided by 0.85
to correct for 15% tissue shrinkage (10). Colonic sections were
examined and imaged in a similar manner. The thickness of the
muscle layers was measured. The external diameter of the empty
colon was evaluated using the external circumference of the freshly
dissected colon.

For myeloperoxidase staining, the colon was opened along the
mesentery and pinned in a dish, and the mucosa and submucosa were
removed under microscopic observation. The segments were fixed in
100% ethanol for 15 min and then washed twice in PBS. Myeloper-
oxidase-positive cells were detected by incubating the tissues in 0.5
mg/ml Hanker-Yates reagent (Sigma) and 5 µl/ml of 3% H₂O₂ in PBS
at room temperature for 12 min (31). Tissues were air dried and
evaporated. Stained cells were counted in seven randomly selected
areas in each specimen.

Assessment of visceral pain. Von Frey hairs were used to measure
the withdrawal responses in unrestrained mice to mechanical stimu-
lation of unshaved skin in the low abdomen. Before the behavioral
tests, the animals were allowed to accustom to the new environment
for at least 30 min. Appearance of the following behaviors on
application of a hair was considered as a withdrawal response: 1) sharp
abdominal retraction, 2) immediate licking or scratching of the
site of application of the hair, or 3) jumping. Hairs calibrated for
forces of 0.5, 1.0, 2.0, 3.0, and 4.0 g were applied 10 times each in
ascending order of force. The probability and threshold of withdrawal
responses were recorded. The hair was applied for 1–2 s at 5- to 10-s
intervals. Care was taken not to stimulate the same point in succes-
sive trials. Statistical analysis. Values are expressed as means ± SE. Fisher’s
exact test, Mann-Whitney test, paired t-test, and ANOVA were used
for comparison. P < 0.05 was considered as statistically significant.

RESULTS

Retrograde labeling of DRG neurons. Two weeks after the DiI
injection into the distal colon, there were numerous DiI-
labeled neurons evenly distributed in DRG L1 (17.6 ± 2.6
cells/ganglion, n = 16) and S1 (17.5 ± 2.8 cells/ganglion), few
in L2 (2.9 ± 1.0 cells/ganglion) and L6 (3.1 ± 1.0 cells/
ganglion), and none in L3, L4, and L5 ganglia. These results are in accord with those obtained by Robinson et al. (30) and indicated that the distal colon receives extrinsic sensory innervation mostly from DRG L1 and S1. We therefore focused on DRG L1 and S1, and L4 ganglia from the same obstructed animals were used as controls.

**Dye coupling.** The dye LY, which crosses gap junctions, was injected into single DRG neurons or SGCs. The labeling process was observed in real time under a fluorescence microscope. All LY-injected neurons \((n = 237)\) were not coupled to other cells, neurons, or SGCs (Fig. 1A), and, also, all LY-injected SGCs were not coupled to any neurons. Glial cells displayed two types of dye coupling: between SGCs enveloping the same neuron (Fig. 1B) and between SGC envelopes around different neurons (Fig. 1C). In control ganglia, the dye coupling incidence was \(2.3\% (n = 258)\) between SGC envelopes and \(22.5\%\) within the SGC envelope around a given neuron. After the induction of obstruction, the incidence of coupling between SGC envelopes was \(7.4\% (n = 231, P < 0.05\) by Fisher’s exact test) at 4 days and \(13.0\% (n = 324, P < 0.001)\) at 6–8 days (Fig. 1D), i.e., the coupling incidence increased \(3.2\)- and \(5.6\)-fold compared with controls. The incidence of coupling between SGCs around the same neuron was \(32.0\% (P < 0.01)\) and \(41.0\% (P < 0.001)\) at 4 and 6–8 days, i.e., increased by \(42\%\) and \(82\%\), respectively (Fig. 1E). The number of SGCs coupled to LY-injected cells increased from \(2.15 ± 0.26\) to \(3.16 ± 0.30\) SGCs/cell \((n = 74, P < 0.05\) by Mann-Whitney test) at 4 days and \(4.15 ± 0.32\) SGCs/cell \((n = 133, P < 0.01)\) at 6–8 days, respectively (Fig. 1F). Dye coupling in L4 from obstructed animals was very similar to that observed in DRG L1 and S1 from sham-operated mice.

To test whether the increased dye coupling after obstruction was due to gap junctions, we added octanol (1 mM) to the bathing solution. In the presence of octanol, the incidence of dye coupling in ganglia from obstructed animals at 6–8 days decreased from \(13.0\%\) to \(1.1\% (n = 88, P < 0.001\) by Fisher’s exact test) between SGC envelopes and from \(41.0\%\) to \(18.6\% (P < 0.001\) between SGCs around the same neuron (Fig. 1, D–F). The results indicate that dye coupling was mediated by gap junctions.

The results described above were obtained when LY was injected randomly into SGCs. When LY was injected into SGCs near (\(< 20\) μm) DiI-labeled neurons, the interenvelope dye coupling in the ganglia of obstructed animals was \(44.1\% (P < 0.0001, n = 59)\) of the LY-injected cells versus \(2.5\% (n = 40)\) in the control, a 18-fold increase. Coupling within envelopes increased from \(25\%\) to \(81.4\%\) after 6–8 days of obstruction (Fig. 2). These results indicated that the response to obstruction occurred preferentially in SGCs located in the vicinity of neurons innervating the obstructed colon.

**Electrophysiological characteristics of DRG neurons.** Intracellular recordings were made from 425 sensory neurons that were impaled randomly and 99 DiI-labeled neurons that projected to the colon. The electrophysiological properties of the

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![Image](https://via.placeholder.com/150)

**Fig. 1.** Dye coupling of dorsal root ganglia (DRG) cells. In control ganglia, a Lucifer yellow (LY)-injected neuron was not coupled to other cells (A), and dye coupling was seen frequently (22.5%) between satellite glial cells (SGCs) ensheathing the same neuron (B), but was only seen in 2.3% of the cases between SGCs of neighboring neurons. After obstruction, the incidence of coupling between SGCs around different neurons \((C)\) increased to 13.0%. \(^{*}\)LY-injected cells; arrows indicate the cell body of SGCs coupled to the dye-injected cells. Scale bars = 20 μm. The histograms show changes after obstruction in coupling incidence between SGC envelopes around different neurons \((D)\), between SGCs around the same neuron \((E)\), and the number of SGCs coupled to the LY-injected cells \((F)\). Fisher’s exact test \((D\) and \(E)\) and Mann-Whitney test were used for comparison. \(^{*}\)\(P < 0.05\) and \(^{**}\)\(P < 0.01\) compared with the control.
cells, respectively) in neurons projecting to the distal colon, as obstructed animals (46% and 42% of controls in A- and C-type randomly). The threshold current was considerably lower in lower in the obstructed animals (in both cell types, impaled neuronal excitability and found that this current was about 30% lower in both A- and C-type neurons in controls (Table 1). We used the membrane input resistance was lower in both A- and C-type neurons (8).

After obstruction, the resting potential was less negative and the membrane input resistance was lower in both A- and C-type neurons compared with controls (Table 1). We used the current threshold for firing an action potential as a measure for neuronal excitability and found that this current was about 30% lower in the obstructed animals (in both cell types, impaled randomly). The threshold current was considerably lower in obstructed animals (46% and 42% of controls in A- and C-type cells, respectively) in neurons projecting to the distal colon, as determined by DiI labeling (see Table 1). Thus partial colonic obstruction augmented neuronal excitability markedly.

In control ganglia, the majority of neurons were electrically quiescent (Fig. 3A; 20.4% (n = 98) of A-type cells and 22.7% (n = 95) of C-type cells displayed subthreshold membrane potential oscillations (Fig. 3B and C). In the obstructed animals, the proportion of neurons displaying subthreshold membrane potential oscillations was more than twofold greater than in controls (P < 0.05 by Fisher’s exact test) in both A- and C-type cells when the neurons were impaled blindly. In neurons projecting into the colon, the proportion was over threefold greater and reached 54.2% and 68.8% in A- and C-type neurons, respectively (P < 0.01). Spectral analysis of the oscillations showed that the peak frequency of A-type neurons was greater than that of C-type cells in control and after obstruction. In control animals, the oscillation frequency was 35.2 ± 3.1 Hz (n = 5) in A-type cells versus

Table 1. Electrophysiological properties of DRG neurons after partial colonic obstruction

<table>
<thead>
<tr>
<th></th>
<th>Control (L1/S1)</th>
<th>Obstructed (L4)</th>
<th>Obstructed (L1/S1)</th>
<th>Control (DiI, L1/S1)</th>
<th>Obstructed (DiI, L1/S1)</th>
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</thead>
<tbody>
<tr>
<td><strong>A-type neurons</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>n</td>
<td>98</td>
<td>47</td>
<td>93</td>
<td>33</td>
<td>24</td>
</tr>
<tr>
<td>RMP, mV</td>
<td>53.0±1.2</td>
<td>54.0±1.4</td>
<td>48.2±1.4*</td>
<td>53.8±1.2</td>
<td>47.5±1.5*</td>
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<tr>
<td>APA, mV</td>
<td>61.8±1.7</td>
<td>61.3±1.2</td>
<td>59.5±1.8</td>
<td>64.2±1.9</td>
<td>61.9±2.0</td>
</tr>
<tr>
<td>APD, ms</td>
<td>6.4±0.2</td>
<td>6.3±0.2</td>
<td>6.4±0.2</td>
<td>6.2±0.3</td>
<td>6.3±0.3</td>
</tr>
<tr>
<td>k, nA</td>
<td>0.47±0.03</td>
<td>0.48±0.03</td>
<td>0.32±0.03†</td>
<td>0.48±0.04</td>
<td>0.26±0.03†</td>
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<tr>
<td>R, MF1</td>
<td>45.5±0.8</td>
<td>47.5±1.2</td>
<td>41.5±0.8*</td>
<td>44.6±1.2</td>
<td>40.2±0.8*</td>
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<tr>
<td>SPS</td>
<td>3 (3.1%)</td>
<td>1 (2.1%)</td>
<td>14 (15.1%)†</td>
<td>1 (3.0%)</td>
<td>6 (25%)†</td>
</tr>
<tr>
<td>SPO</td>
<td>20 (20.4%)</td>
<td>10 (21.3%)</td>
<td>40 (43.0%)†</td>
<td>5 (15.1%)</td>
<td>13 (54.2%)†</td>
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<tr>
<td><strong>C-type neurons</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>n</td>
<td>75</td>
<td>35</td>
<td>77</td>
<td>29</td>
<td>32</td>
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<tr>
<td>RMP, mV</td>
<td>53.5±1.5</td>
<td>53.6±1.4</td>
<td>48.0±1.4*</td>
<td>54.5±2.1</td>
<td>49.1±1.2*</td>
</tr>
<tr>
<td>APA, mV</td>
<td>62.8±1.9</td>
<td>61.4±1.7</td>
<td>59.8±1.8</td>
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<td>62.6±2.0</td>
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<td>k, nA</td>
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<td>0.41±0.04</td>
<td>0.29±0.02†</td>
<td>0.38±0.03</td>
<td>0.22±0.03†</td>
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<td>R, MF1</td>
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<td>47.3±1.2</td>
<td>42.4±0.8*</td>
<td>45.8±1.2</td>
<td>42.6±1.1*</td>
</tr>
<tr>
<td>SPS</td>
<td>2 (2.7%)</td>
<td>1 (2.9%)</td>
<td>14 (18.2%)†</td>
<td>1 (3.4%)</td>
<td>9 (28.1%)†</td>
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<tr>
<td>SPO</td>
<td>17 (22.7%)</td>
<td>7 (20%)</td>
<td>36 (46.8%)†</td>
<td>6 (20.7%)</td>
<td>22 (68.8%)†</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n is the number of neurons examined. RMP, resting membrane potential; APA, action potential amplitude; APD, action potential duration; k, current threshold for firing an action potential; R, membrane input resistance; SPS, spontaneous spikes; SPO, spontaneous membrane potential oscillations. *P < 0.05 and †P < 0.01 compared with controls. Fisher’s exact test and Mann-Whitney test were used for comparison.
24.5 ± 3.3 Hz (n = 6) in C-type cells (P < 0.05 by Mann-Whitney test). After obstruction, the frequency was 29.7 ± 2.1 Hz (n = 13) in A-type neurons and 24.2 ± 1.7 Hz (n = 22) in C-type neurons (P < 0.05; see Fig. 3, F and G). Qualitatively similar results were obtained by Amir et al. (1) in rat DRG neurons after axotomy.

Neurons with spontaneous action potentials (Fig. 3, D and E) were rare in control ganglia (3.1% of A-type cells and 2.7% in C-type cells). After obstruction, the proportion of neurons firing spontaneously increased by over fivefold in randomly impaled A- and C-type neurons and by over eightfold in neurons of both types projecting into the distal colon (P < 0.01 compared with the control).

Change in neuronal size. Figure 4A shows a cross section of a control ganglion, and Fig. 4B shows a ganglion from an obstructed animal. The neurons appeared to be larger after obstruction, and this was demonstrated quantitatively in the size distributions shown in Fig. 4C. The mean cross-sectional area of neurons in obstructed animals was 35.3% greater than the control (control, 1,122.1 ± 33.9 μm², n = 318; obstruction, 1,518.1 ± 34.3 μm², n = 337; P < 0.0001 by Mann-Whitney test; see Fig. 4D).

Changes in the colon. The thickness of the muscle layers in the obstructed colon was greater than that in the control by 60% (P < 0.0001 by Mann-Whitney test; Fig. 5), and the diameter of the obstructed colon was greater than that in the control by 69.8% (P < 0.0001 by Mann-Whitney test; Fig. 5D). Myeloperoxidase staining showed that the density of neutrophils in the colonic muscle layers was 89.2 ± 6.4 cells/mm² in the controls versus 406.9 ± 36.0 cells/mm² after obstruction (n = 4 for each group, P < 0.0001 by Mann-Whitney test; see Fig. 6). These results show that obstruction led to hypertrophy and inflammation of the colon.

Change in pain threshold. Before induction of the partial obstruction, the threshold for the withdrawal response was 3.83 ± 0.12 g (n = 18) compared with 1.14 ± 0.12 g after 6
days of obstruction ($P < 0.0001$ by paired $t$-test). Thus the threshold was 70% lower. The probability (in %; ±SE) of the withdrawal response to the mechanical stimulation to the low abdominal skin is summarized in Fig. 7. The probability was significantly higher in the obstructed than the control animals. These findings indicate that obstruction induced hyperalgesia of the somatic area to which visceral pain from the colon referred.

Our results demonstrate that partial colonic obstruction induces profound physiological and morphological changes in neurons and SGCs in DRG that innervate the colon. Thus intestinal lesion can lead to changes not only locally but also at far removed sites, as found for DRG neurons after intestinal (3,
23) or gastric inflammation (4) and ulcers (5, 6). We investigated only the DRG, but there is evidence that GI injury may have long-term effects on the central nervous system as well (24). Therefore, the behavioral changes we observed may be due to sensitization at the DRG level combined with a central contribution. The DISCUSSION below is an attempt to integrate the morphological, physiological, and behavioral observations.

Dye coupling among SGCs. In control ganglia, dye coupling was rather common (22.5%) among SGCs ensheathing a given neuron and rare among SGCs forming envelopes around different neurons. After obstruction, coupling between glial envelopes increased about 4-fold when the cells were injected randomly and 18-fold when the injected SGCs were close to neurons projecting to the distal colon. These changes were observed only in DRG L1 and S1, which were found by us and others (30) to project to the colon. These observations indicate that the cellular changes in DRG are related to events occurring in the obstructed colon.

Dye coupling was largely inhibited by the gap junction blocker octanol, indicating that it was due to gap junctions connecting the SGCs. The morphological basis for the increased coupling is not yet known, but it can be proposed that it is at least partly the result of growth of glial processes bridging SGCs around different neurons and the formation of new gap junctions between them, as found in our electron microscopic study on DRG after axotomy (17, 27). The functional significance of the augmented glial coupling is not clear, but two possible ideas can be put forward. First, it is established that in the central nervous system, glial coupling contributes to the buffering of K\(^{+}\) that accumulate in the extracellular space during intense neural activity (25). There is evidence for ectopic electrical activity in DRG neurons after peripheral nerve injury (11), as also found in the present study (Fig. 3), which may lead to local K\(^{+}\) accumulation. It can be proposed that the augmented glial coupling after obstruction prevents the build up of harmful levels of K\(^{+}\) in the ganglia. Interestingly, increasing extracellular K\(^{+}\) was found to promote dye coupling between SGCs (20). Second, it is known that damage causes tissues to revert to an embryonic-like state, which is characterized by extensive intercellular coupling via gap junctions (13, 22). It is conceivable that during obstruction-induced damage, the same mechanism is activated. We proposed previously that the increased glial coupling after nerve injury might contribute to the symptoms of neuropathic pain, as it has the potential to mediate long-distance communications between neurons (9, 17). A similar mechanism may operate during obstruction.

Changes in DRG neurons. We found that the cross-sectional area of neurons in DRG that innervate the partly obstructed colon was 35.3% greater than that in controls. Similar results were reported for rat DRG after partial obstruction of the ileum (42), rat pelvic ganglion after partial obstruction of the urinary bladder outlet (15), and in rat myenteric plexus after partial obstruction of the ileum (14). In all these cases, there was also hypertrophy of smooth muscle in the obstructed organs. Purves (28) proposed that the size of neurons increases when their target organs undergo hypertrophy, and apparently the same principle applies to the cases mentioned above as well as to the present results. A possible explanation for this phenomenon is the greater metabolic demand on the neurons innervating the hypertrophic muscles (26). Another factor that probably con-
tributed to the neuronal changes is the inflammation in the colon that was associated with the partial obstruction.

We believe that this is the first study of changes induced by obstruction on the physiological properties of DRG neurons. Using intracellular recordings, we showed that the threshold for firing a spike was lower in neurons in DRG L1 and S1 after obstruction. Also, the number of neurons displaying spontaneous subthreshold potential oscillations and the number of neurons firing spontaneous action potentials were significantly higher in the obstructed animals. All these effects are consistent with increased excitability of the neurons. Augmented excitability of sensory neurons was observed after inflammation in the stomach (4), ileum (23), and colon (3). These reports provided evidence that increased expression of TTX-resistant Na\(^+\) channels may underlie this effect. Similar physiological changes were observed in DRG after anatomy of the sciatic nerve (44), and Amir et al. (1) proposed that the excitability changes were associated with an increase in the amplitude of spontaneous potential oscillations. They also found that the amplitude of these oscillations was enhanced by depolarization (2), which may be correlated with our observation that in the obstructed animals DRG neurons were depolarized by an average of 4–6 mV compared with controls.

The proportion of DRG neurons affected by obstruction is very likely to be greater than the number of DRG neurons projecting to the distal colon. There is no accurate information on the number of neurons innervating the colon, but an estimate can be obtained from retrograde labeling studies. In the rat colon, 1–1.2% of DRG S1 neurons were found to innervate the colon (35, 36), and the numbers are expected to be similar in the mouse colon. We found in both dye coupling and electrical recordings that over 20% of the DRG neurons and SGCs were affected by the obstruction, indicating that the signals coming from the affected colon spread in the ganglion. This implies that the affected neurons influence a much larger population of neurons in the ganglion. As found in the dye coupling experiments, glial cells were also affected by the colonic obstruction. This includes not only SGCs around affected neurons but also glia around many of the unaffected ones, again indicating the presence of long-range influences. The mechanisms underlying these cellular changes are still unknown. We propose that augmented glial coupling has an important role in the spread of the augmented neuronal excitability. Evidence for long-range effects within sensory ganglia after injury has been obtained by several authors (21, 29, 32). The nature of the signals mediating this spread is not known, but it can be suggested that chemical messengers released from the affected neurons travel within the ganglia, thus altering cellular properties. Possible candidates can be nitric oxide or nerve growth factor, which are produced at increased rates after nerve damage (6, 37, 38). It is established that in neuropathic pain states, there is a spread of the sensation beyond the injured neuron (11). The present results are consistent with this concept and provide ideas for possible mechanisms that can account for it.

**What are the mechanisms underlying the cellular changes?**

We consider the evidence for inflammatory processes in the partly obstructed colon as a key observation because it appears to account for most of the present findings. In a study (43) on the inflamed urinary bladder, it was found that the respective DRG neurons were hypertrophied, which was explained by the release of inflammatory mediators and/or neurotrophins. Because the release of these agents appears to be a general phenomenon in inflamed visceral organs in both humans (12) and animals (6, 34), it can be proposed that retrograde transport of these substances into DRG contributes to the hypertrophy of DRG neurons. Thus both the presence of inflammation and the thickening of the colonic wall that resulted from partial colonic obstruction may have contributed to the neuronal hypertrophy reported here. We propose that colonic inflammation served as a major trigger for the other observed changes in the DRG cells. This hypothesis is supported by our recent experiments (18), which showed that chemically induced colonic inflammation led to similar effects as partial colonic obstruction.

**Partial colonic obstruction and abdominal pain.** Ectopic firing of sensory neurons was proposed as an important contributing factor in neuropathic pain (11). Similarly, the augmented excitability and spontaneous electrical activity observed in sensory neurons innervating the obstructed colon might contribute to visceral pain. GI inflammation (3, 4, 23) augmented neuronal excitability, which was proposed to contribute to abdominal pain. Previous studies did not consider the role of SGCs in visceral sensation, but our observations on the increased coupling among these cells indicate that inflammation-induced changes in SGCs should be taken into account along with altered neuronal excitability when neuropathic pain is discussed. It can be hypothesized that glial changes are involved in events leading to neuronal sensitization. Because this coupling is mediated by gap junctions, it can be proposed that drugs that block gap junctions may have a therapeutic potential as analgesics for visceral pain, especially as there is recent progress in developing such drugs (33). Furthermore, SGCs were found to express a variety of receptors (19), which may serve as drug targets; we have shown that SGCs possess receptors for ATP (41), which has a role in pain signaling (16). This and other mechanisms are currently under research in our laboratory.

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