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# The Location of Extrinsic Afferent and Efferent Neurons Innervating The Stomach and Colon in Rat

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**Abstract:** The location of extrinsic afferent and efferent neurons projecting to the rat gastrointestinal tract were mapped using the retrograde tracer, True Blue (TB). Injection of retrograde tracer, TB into stomach, proximal colon and distal colon resulted in labelled cells in the dorsal root ganglia. Cells were also labelled in the nodose ganglia following injection of tracer into stomach and proximal colon.

TB-labelled cells were also seen in the prevertebral ganglia (coeliacomesenteric complex and inferior mesenteric ganglion), after injection of TB into the stomach, proximal and distal colon. Many TB-labelled cells were detected in the dorsal motor nucleus of the vagus (DMNX) following injection of tracer into stomach. Labelled cells were less numerous following injection of tracer into the distal colon.

Key Words: Retrograde tracer, afferent neuron, efferent neuron, stomach, colon , rat.

## Ratlarda Mide ve Kolonu İnnerve Eden Ekstrinsik Afferent ve Efferent Nöronların Lokalizasyonu

**Özet:** Ratlarda mide ve kolona uzantı gönderen ekstrinsik afferent ve efferent nöronların lokalizasyonu retrograde tracer, True Blue (TB) kullanılarak araştırıldı. Tracer'in mide ile kolonun başlangıç ve son bölümlerine enjeksiyonu sonrası spinal ganglionda TB ile işaretlenmiş hücrelere rastlandı. Tracer'in mide ve kolonun başlangıcına enjeksiyonu sonrası vagal ganglionda işaretlenmiş hücreler gözlendi.

TB'nin mide, kolonun başlangıç ve son bölümlerine enjeksiyonunu takiben prevertebral ganglionda (coeliacomesenteric complex ve inferior mesenteric ganglion), TB ile işaretlenmiş hücrelere rastlandı. Tracer'in mide'ye enjeksiyonu sonrası çok sayıda, kolonun başlangıç kısmına enjeksiyonu sonrası ise vagusun dorsal motor nükleusunda (DMNX) az sayıda işaretlenmiş hücreler gözlendi. Tracer'in distal kolona enjeksiyonu sonucu ise DMNX ve sakral medulla spinalis'de TB ile işaretlenmiş hücrelere rastlanmadı.

Anahtar Sözcükler: Retrograde tracer, afferent nöron, efferent nöron, mide, kolon, rat.

## Introduction

The innervation of the gastrointestinal tract consists of a vast number of nerve cell bodies and their processes embedded in the wall of the gut, together with an input from extrinsic sources (1-4).

Early physiological studies clearly demonstrated the presence of extrinsic fibres projecting to the gut (5-12). However, precise identification of the cells involved awaited the development of retrograde tracing techniques, involving the pinocytotic uptake of a substance at nerve terminals, its transport back to the cell body, and its subsequent visualisation (13-21).

In the present study, the origins of afferent and efferent fibres projecting to stomach, proximal and distal colon were examined using fluorescence retrograde tracing technique.

## Materials and Methods

### Retrograde tracing techniques

Adult twelve wistar rats were anaesthetised with hypnorm (0.3 ml/kg, i.m.) and diazepam (2.5 mg/kg, i.p.). Using aseptic techniques suspension of True Blue in distilled water (5% w/v) was injected into the ventral wall of stomach, into the proximal colon or into the distal colon with 10 µl Hamilton microsyringe. A total of 20 µl, in volumes of 1-2 µl, was injected into each region. After each injection, the needle was left in place for up to 1 minute to reduce leakage of dye long the needle tract, and injection site was then swapped with saline. Viscera were replaced in the abdominal cavity, and the incision in the abdominal muscle, then skin was sutured.

### Tissue processing

Seven days after injection, rats were terminally

Table 1. Percentage of True Blue-labelled spinal afferents (mean±S.D.), after injecting TB into the stomach (left and right DRGs, segments T9-$L_3$, 3 rats).

| Ganglia | % |
|---|---|
| $T_9$ | 24.1±4.7 |
| $T_{10}$ | 19.8±1.6 |
| $T_{11}$ | 24.7±2.5 |
| $T_{12}$ | 17.1±2.1 |
| $T_{13}$ | 9.9±6.7 |
| $L_1$ | 4.1±2.4 |
| $L_2$ | 1.2±0.1 |
| $L_3$ | 0.8±0.1 |

Table 2. Percentage of True Blue-labelled spinal afferents (mean±S.D.), afternjecting TB into the proximal colon (left and right DRGs, segments $T_{10}$-$S_3$, 6 rats).

| Ganglia | % |
|---|---|
| $T_{10}$ | 19.5±3.8 |
| $T_{11}$ | 26.3±6.5 |
| $T_{12}$ | 22.4±1.9 |
| $T_{13}$ | 21.4±3.6 |
| $L_1$ | 8.1±3.3 |
| $L_2$ | 2.5±1.9 |
| $L_3$ | 0.8±0.1 |
| $L_4$ | 0 |
| $L_5$ | 0 |
| $L_6$ | 0 |
| $S_1$ | 0 |
| $S_2$ | 0 |
| $S_3$ | 0 |

anaesthetised (overdose of sodium pentobarbitone, i.p.) and 0.1 ml of heparin (5000 I.U. per ml) was injected into the left ventricle of the heart. The animals were transcardically perfused with PBS (0.1M phosphate buffer, 0.14M sodium chloride, pH 7.4) followed by 4% formaldehyde in sodium cacodylate buffer (0.1M, pH 7.4).

Nodose ganglia, dorsal root ganglia, brain stem, spinal cord (segment T9-10 and S1-2), celiac, splanchnic and inferior mesenteric ganglia were removed. Tissues were placed in 0.1M sodium cacodylate, pH 7.4, containing 20% sucrose for at least 24 hours at 4°C before snap freezing and sectioning at 10-15μm in a cryostat. Sections were examined using a Leitz Dialux 20 microscope. Labelled cells were observed using Leitz excitation-emission filter pack A for True Blue.

## Results

### Extrinsic afferents

Injection of retrograde tracer, TB into stomach, proximal colon and distal colon resulted in labelled cells in the dorsal root ganglia. Cells were also labelled in the nodose ganglia following injection of tracer into stomach and proximal colon (Figs. 1 & 2).

### Gastric afferents

In dorsal root ganglia, TB labelling was bilateral and, in any one segment, left and right ganglia contained similar number labelled cells. Higher numbers were seen in ganglia of segments $T_9$-$T_{12}$, the number of labelled cells declined rapidly in lumbar ganglia (Table 1).

Labelling in the nodose ganglia was also bilateral, but the left ganglia contained more TB-labelled cells than the right. TB-labelled cells were concentrated around the caudal pole of the ganglion.

### Intestinal afferents

Spinal projections to the proximal colon extended mainly from $T_{10}$ through to $L_2$. Highest numbers of TB-labelled cells were seen in ganglia of segments $T_{10}$-$T_{13}$, numbers of labelled cells declined rapidly in lumbar segments (Table 2).

Vagal afferents projecting to the proximal colon were detected bilaterally, they were much fewer in number compared to vagal gastric afferents.

Spinal projections to the distal colon werefound mainly in the thoracolumbar segments $T_{11}$-$L_2$, and in the first sacral segment, with the majority of labelled cells lying in segments $L_1$-$L_2$ and $S_1$ (Table 3). No vagal projection to the distal colon could be detected.

### Extrinsinc efferents

Injections of TB into the stomach, proximal and distal

Table 3. Percentage of True Blue-labelled spinal afferents (mean ±S.D.), after injecting TB into the distal colon (left and right DRGs, segments $T_{10}$-$S_3$, 6 rats).

| Ganglia | % |
|---|---|
| $T_{11}$ | 10.9±5.9 |
| $T_{12}$ | 9.2±2.3 |
| $T_{13}$ | 9.1±1.3 |
| $L_1$ | 25.8±2 |
| $L_2$ | 21.1±9.7 |
| $L_3$ | 1.3±0.8 |
| $L_4$ | 0 |
| $L_5$ | 0 |
| $L_6$ | 0 |
| $S_1$ | 21.5±9.5 |
| $S_2$ | 0 |
| $S_3$ | 0 |

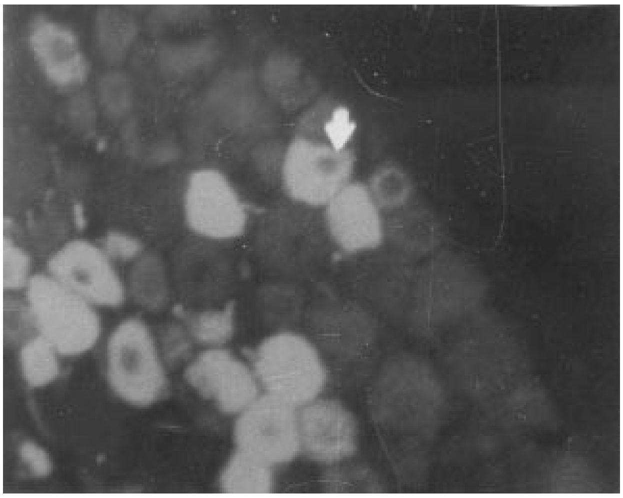

Figure 1. TB labelled cells in dorsal root ganglion from a rat injected with TB in the ventral wall of the stomach. 230x.

colon resulted in TB-labelled cells in the prevertebral ganglia, that is, the coeliacomesenteric complex and inferior mesenteric ganglion (Fig. 3).

Following injection of TB into stomach and proximal

Figure 2. TB labelled cells in nodose ganglion from an rat injected with TB in the wall of the proximal colon. 230x.

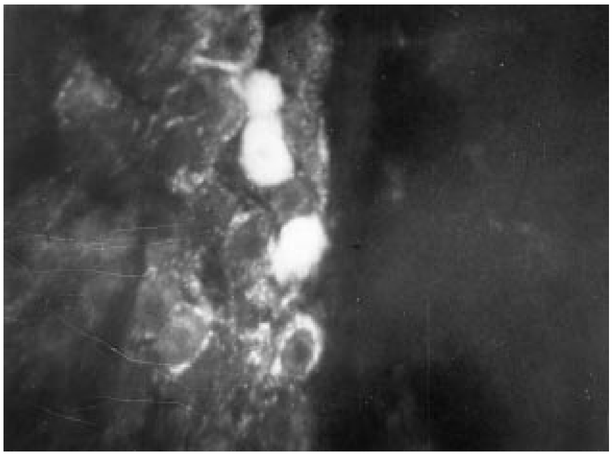

Figure 3. TB labelled cells in inferior mesenteric ganglion from a rat injected with TB in the wall of the distal colon. 300x.

colon, there was a distinctive distribution of labelled cells in the coeliacomesenteric complex. Within the fused coeliacomesenteric ganglia, moderate numbers of labelled cells were scattered throughout the tissue. However, as the splanchnic ganglia, moderate numbers of labelled cells were scattered throughout the tissue. However, as the splanchnic nerves approached the coeliacomesenteric ganglion, there were numerous cell bodies in the nerve trunks of which a large proportion were labelled. In the text these regions are termed the splanchnic ganglia, whereas the remaining part of the complex, the coeliacomesenteric ganglion, is abbreviated to coeliac ganglion.

Following injection of tracer into the stomach, labelled cells were found mainly in the splanchnic and coeliac

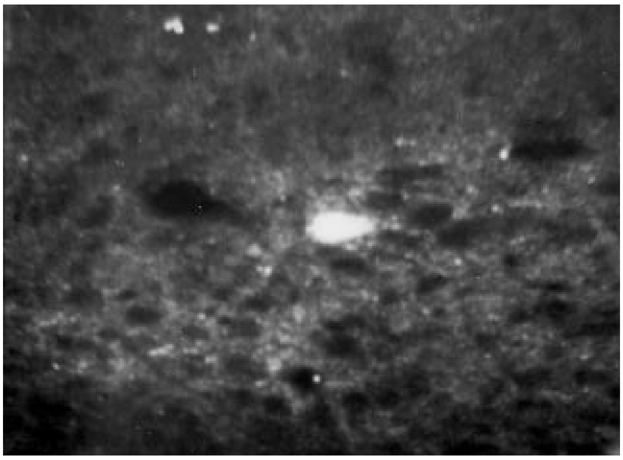

Figure 4. TB labelled cells in dorsal motor nucleus of the vagus (DMNX) of a rat injected with TB in the wall of the proximal colon. 300x.

ganglia. Very few cells were detected in the inferior mesenteric ganglion. Injection of tracer into the distal colon resulted in labelling of cells mainly in the coeliac and inferior mesenteric ganglion, with few cells labelled in the splanchnic ganglia, whereas after injection into the proximal colon labelled cells were found in all three tissues.

Many TB-labelled cells were detected in the dorsal motor nucleus of the vagus (DMNX) following injection of tracer into stomach. Labelled cells were less numerous following injection of tracer into the proximal colon (Fig. 4).

Almost no labelled cells were seen in the DMNX or sacral spinal cord following injection of tracer into the distal colon.

## Discussion

### Afferent neurons

The distribution of gastric afferents in dorsal root ganglia observed in the present study is in agreement with earlier reports of spinal afferents projecting to the stomach of the rat, guinea pig and cat (14-18). Similarly, the restriction to the caudal pole of the nodose ganglion of vagal afferents projecting to the stomach has been reported previously (22, 23).

In the present study, a different pattern was seen in the sensory projections to proximal and distal colon. Spinal projections to the proximal colon were found mainly in thoracic segments $T_{10}$-$T_{13}$, whereas those projecting to the distal colon were found mainly in lumbar segments $L_1$ and $L_2$ and in the first sacral segment.

This distribution of spinal afferents projecting to the distal colon is in agreement with data from other studies on the rat (18, 19). Baron and Janig (24) showed that application of tracer to lumbar colonic nerves labelled cells in dorsal root ganglia mainly in lumbar segments $L_1$ and $L_2$. Thus, it appears that most of the spinal afferents projecting to the distal colon do so *via* the inferior mesenteric ganglion and lumbar colonic nerves, whereas those projecting to the proximal colon clearly take a different route.

A substantial number of cells in the coeliac complex project to the proximal colon (this study, 25) *via* the inferior coeliac nerves (25), and it is probable that primary afferents projecting to the proximal colon from thoracic ganglia follow this more cranial route.

Although, Altschuler et al. (20) detected labelled afferent nerve terminals in the nucleus of the solitary tract following injection of retrograde tracer into the rat colon, no study until now has demonstrated nodose ganglion cells projecting to the colon. In the present sutdy, labelled cells were detected bilaterally following injection into the proximal colon, but they wereuch fever in number than vagal afferents projecting to the stomach. In the study by Altschuler et al. (20), labelled fibres were detected in the nucleus of solitary tract in one out of five animals that had received injection of cholera toxin-peroxidase tracer into the distal (descending) colon. In contrast, in the present study, no vagal afferents projecting to the distal colon could be detected, which is probably due to difference in the retrograde tracer employed or to reflection of the more distally restricted site of injection used here.

### Efferent neurons

As observed in the present study, retrograde labelling studies in various species have demonstrated that the stomach in innervated by parasympathetic pregnanglionc neurons from the dorsal motor nucleus of the vagus (DMNX) (14, 15, 18, 26-28). The DMNX also projects to the proximal colon as has been previously shown in rat (20), cat (13) and dog (29), with a marked decline in the number of cells projecting to distal colon.

Although labeled cells have been detected in the sacral spinal cord after injection of tracer into the distal region of colon in mouse (30), cat (13), rabbit (31) and dog (29), extremely few cells were detected in the present study on the rat, suggesting only a limited parasympathetic innervation of this region. In support of this, Altschuler et al. (20) found very few labelled cells in the sacral spinal cord in only one out of five rats receiving injection of cholera toxin-peroxidase tracer into the distal colon and relatively few labelled cells in the DMNX. An alternative explanation for absence of label in sacral preganglionic parasympathetic neurons is that these noeurons project to the distal colon largely *via* second order neurons in pelvic ganglia, as is seen in the dog (29), rather than projecting directly to the gut. It is not known if these ganglia exist in the rat. In the dog they could not be identified at dissection, and were only recognised at light microscopic level by the presence of labelled cells. Further retrograde labelling studies are required to explore this posibility.

It is well established that the coeliac ganglion sends projections mainly to the stomach and small intestine, with a minor projection to the proximal colon, whereas the large intestine receives its sympathetic innervation mainly from the inferior mesenteric ganglion (18, 23, 25). The results of the present study are in general agreement with there fidings, but include evidence of projections from the splanchnic ganglia to stomach and colon.

In an early report, Green (32) described the coeliac complex as a single ganglion, but later studies revealed multiple components, namely suprarenal, aorticorenal and coeliac ganglia, but no separate superior mesenteric ganglia (33, 34, 35). The splanchnic ganglia described in the present study are identical in position to the suprarenal ganglia, so named by Baljet and Drukker (33) primarily on the grounds of their anatomical position, although they did stress projections from the ganglia to the adrenal glands. Similar ganglia in man have been described as splanchnic ganglia of Lobstein (36). While these ganglia within the splanchnic nerves may project to the adrenal glands, the present study has demonstrated that they have a much wider territory, that is, the gastrointestinal tract. Thus, it seems more appropriate to apply the term ‘splanchnic’, rater than ‘suprarenal’, to these ganglia.

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