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# Activation in vagal afferents and central autonomic pathways: Early responses to intestinal infection with *Campylobacter jejuni*

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**Abstract**

Abundant evidence now supports the idea that multiple pathways or mechanisms underlie communication from the immune system to the brain. The presence of a variety of mechanisms suggests that they may each contribute something different to immunosensory signaling. For instance, brain mediated immune signal transduction is dependent upon the presence of circulating mediators whereas peripheral sensory nerves are more likely to be important early on in an infection, prior to elevation of circulating cytokines, or in local infections within the terminal fields of these nerves. To test the hypothesis that local infection in the gut activates vagal sensory neurons, we assessed expression of the neuronal activation marker c-Fos in neurons in the vagal sensory ganglia and in the primary sensory relay nucleus for the vagus, the nucleus of the solitary tract (nTS) in mice treated orally either with saline or live *Campylobacter jejuni* (*C. jejuni*). Male CF1 mice were inoculated orally with either *C. jejuni* or saline, and c-Fos expression in the vagal sensory neurons and brain 4–12 h later was assessed via immunohistochemistry. Oral inoculation with *C. jejuni* led to a significant increase in c-Fos expression in neurons bilaterally in the vagal ganglia, in the absence of elevated levels of circulating pro-inflammatory cytokines. *C. jejuni* treatment activated neurons in the nTS, as well as in brain regions associated with primary viscerosensory pathways and the central autonomic network. These findings provide evidence that peripheral sensory neurons contribute an early signal to the brain regarding potential pathogens.


*Keywords:* Gram-negative bacteria; Vagal afferents; c-Fos; Nucleus of the solitary tract; Paraventricular hypothalamus; Gut–brain interaction

## 1. Introduction

Peripheral immune activation induces brain-mediated functions keyed to supporting host defense including thermogenesis, neuroendocrine responses, as well as exerting profound influence on sleep, social behavior, and cognition (Kent et al., 1992; Maier, 2003). Abundant evidence now supports the idea that multiple pathways or mechanisms underlie communication from the immune system to the brain (reviewed in Dantzer, 2001; Goehler et al., 2000; Rivest, 2001). These mechanisms include active transport of cytokines (Banks et al., 2002) into the brain parenchyma, interaction of cytokines with endothelial cells of the brain microvasculature (Ericsson et al., 1997; Yamagata et al., 2001), cytokines, as well as pathogens themselves, interacting with immune cells or neurons in circumventricular organs (Lee et al., 1998; Rivest, 2001), or in barrier tissues such as choroid plexus, ventricular ependyma, and meninges. In addition to interacting with brain tissues directly, cytokines

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activate primary sensory neurons associated with peripheral nerves, notably but not exclusively, the vagus (Ek et al., 1998; Goehler et al., 1998; Romeo et al., 2001; Watkins et al., 1995).

The presence of a variety of mechanisms suggests that they may each contribute something different to immunosensory signaling. Although to date few studies have addressed this issue, a consensus seems to exist regarding the general circumstances in which direct brain-mediated signal transduction occurs compared to the activation peripheral nerves. That is, brain mediated immune signal transduction is dependent upon the presence of circulating mediators (such as cytokines or pathogens/pathogen products), whereas peripheral nerves are more likely to be important perhaps early on in an infection, prior to elevation of circulating cytokines, or in local infections within the terminal fields of these nerves. For example, the vagus nerve innervates most internal organs, including lung and gastrointestinal tract, and thus may signal local infection or inflammation in these tissues.

Whereas the idea that the vagus nerve senses local infection in, for instance, the gut seems plausible, this idea has never been directly tested. Studies investigating the role of vagal sensory nerves in immune-to-brain signaling have utilized bolus injection of immune stimulants, such as bacterial endotoxin, or cytokines. Peripherally injected substances, even low intraperitoneal doses, eventually lead to circulating levels (e.g., Gaykema et al., 2000; Hansen et al., 2001), which may not necessarily reflect the conditions during natural infection or inflammation. Hence, it has been difficult to determine the likely contribution of specific signaling pathways to brain responses to inflammation.

Local infections can lead to subtle changes in affective states and emotional responsiveness (Bradfield et al., 1992; Lyte et al., 1998), as well as the induction of fever and neuroendocrine responses (Campisi et al., 2004). In a model of gastrointestinal infection with *Campylobacter jejuni* (*C. jejuni*), mice developed anxiety-like behavior in the absence of systemic immune activation (Lyte et al., 1998). A subsequent study showed that this local gastrointestinal infection leads to c-Fos induction (used as a measure of neuronal activation) in a subset of central autonomic network brain nuclei also evoked by exogenous administration of immune stimulants. This effect was seen, again, in the absence of obvious systemic immune activation (Gaykema et al., 2004). Thus it is possible that a neural route, such as via vagal sensory nerves, may obtain in this paradigm.

To test the hypothesis that local infection in the gut activates vagal sensory neurons, we directly assessed expression of the neuronal activation marker c-Fos in neurons in the vagal sensory ganglia in mice treated orally either with saline or live *C. jejuni*. We also documented c-Fos patterns in the primary sensory relay nucleus for the vagus, the nucleus of the solitary tract (nTS), as well as induction in higher-order brain regions. *C. jejuni* colonizes a fairly restricted part of the gastrointestinal tract, the cecum, that is innervated by vagal sensory nerve fibers, making it an appropriate model for assessing a role of the vagus in signaling local infection.

Whereas previous experiments investigating effects of *C. jejuni* on the nervous system have assessed responses at time points at which the infection was established (18–42 h) in the cecum, the aims of the experiments described here were focused on detecting more rapid immunosensory events. Based on estimates of intestinal transit time in mice and pilot data (not shown), we chose a survival time window of between 4 and 13 h post-inoculation. The wide time window was used to enhance the likelihood of observing c-Fos expression in vagal sensory neurons, as this can be transient, and to determine latency, or time to onset, of vagal sensory neuron activation following inoculation with *C. jejuni*.

To gain clues to the potential signaling mechanism from the bacteria to vagal sensory fibers, we assessed c-Fos induction in the enteric (local intrinsic) ganglia in the cecum. In addition to innervating the submucosa and subepithelium of the gut, vagal sensory neurons also innervate these enteric ganglia. Neurons in the enteric ganglia express receptors for the cytokine IL-1, and express c-Fos immunoreactivity under conditions of infection or inflammation (Sharkey and Kroese, 2001; Sharkey and Mawe, 2002), and thus may serve as an immunosensory conduit to the vagus. In addition, to assess potential inflammation and cellular infiltration we stained sections of cecum with Cresyl violet. Because our previous study (Gaykema et al., 2004) lacked measures of circulating cytokines at the early time points investigated in this study, we also assessed levels of the cytokines IL-1β, TNFα, and IL-6 between 4 and 8 h post-inoculation, i.e., during the onset of establishment of the bacterial pathogen.

## 2. Materials and methods

### 2.1. Animals

Male CF-1 mice ($n = 66$) were purchased from Charles River (Portage, MI), and upon receipt, maintained on a reversed day-night light cycle with lights off at 4:00 AM and on at 4:00 PM. They were housed in groups of two per cage (polypropylene with a bedding of wood shavings) with water and food available ad libitum, and were allowed to acclimate for at least two weeks. Animals received per oral challenge with *C. jejuni* ($5 \times 10^8$ colony-forming units; $n = 38$) or saline ($n = 28$). All procedures were carried out in accordance with protocols approved by the Institutional Animal Care and Use Committee of the Minneapolis Medical Research Foundation.