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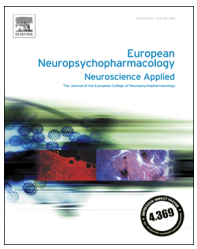

The vagus nerve modulates BDNF expression and neurogenesis in the hippocampus

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Abstract
Accumulating evidence suggests that certain gut microbiota have antidepressant-like behavioural effects and that the microbiota can regulate neurogenesis and the expression of brain-derived neurotrophic factor (BDNF) in the hippocampus. The precise mechanisms underlying these effects are not yet clear. However, the vagus nerve is one of the primary bidirectional routes of communication between the gut and the brain and thus may represent a candidate mechanism. Yet, relatively little is known about the direct influence of vagus nerve activity on hippocampal function and plasticity. Thus, the aim of the present study was to determine whether constitutive vagus nerve activity contributes to the regulation of neurogenesis and BDNF mRNA expression in the hippocampus. To this end, we examined the impact of subdiaphragmatic vagotomy in adult mice on these parameters. We found that vagotomy decreased BDNF mRNA in all areas of the hippocampus. Vagotomy also reduced the proliferation and survival of newly born cells and decreased the number of immature neurons, particularly those with a more complex dendritic morphology. Taken together, these findings suggest that vagal nerve activity influences neurogenesis and BDNF mRNA expression in the adult hippocampus.

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1. Introduction

Accumulating evidence suggests that gut microbiota can influence hippocampal neuronal plasticity and behaviours linked with hippocampal function including antidepressant-like behaviour and cognition (Clarke et al., 2013; Gareau et al., 2011; Luczynski et al., 2016; Ogbonnaya et al., 2015). The mechanisms underlying these effects are not yet fully understood but signalling through the vagus nerve which is one of the primary bidirectional routes of communication between the gut and the brain is a candidate mechanism (Berthoud, 2008; Forsythe et al., 2014). Indeed, the ability of some bacterial strains to reduce depression-like behaviours and to alter the expression of certain proteins in the hippocampus have been shown to be dependent upon the vagus nerve (Bercik et al., 2011b; Bravo et al., 2011). However, relatively little is known about the direct role of the vagus nerve in hippocampal neuronal plasticity.

The neurotrophic factor, brain-derived neurotrophic factor (BDNF) plays a key role in several aspects of hippocampal neuronal plasticity and function (Andero et al., 2014; Lu et al., 2014), and is one of the hippocampal proteins involved in gut-brain signalling. Intriguingly, alterations in BDNF expression have been reported in the hippocampus of germ-free mice (Clarke et al., 2013; Neufeld et al., 2011; Sudo et al., 2004), antibiotic-treated mice (Bercik et al., 2011a) and in rats that received vagus nerve stimulation (VNS) which is used as a treatment for refractory depression (Biggio et al., 2009; Follesa et al., 2007). BDNF plays a critical role in adult hippocampal neurogenesis (Li et al., 2008; Vilar and Mira, 2016), a process that contributes to hippocampal plasticity and that has been implicated in some aspects of hippocampus-dependent cognition and antidepressant action. Intriguingly, adult hippocampal neurogenesis is regulated by the $GABA_B$ receptor (Felice et al., 2012; Giachino et al., 2014; O'Leary et al., 2014), a receptor which has also been associated with the antidepressant-like behavioural effects of the bacterium *Lactobacillus rhamnosus (JB-1)*(Bravo et al., 2011). Moreover, recent studies from our lab and that of others, suggest that the microbiota can also regulate adult hippocampal neurogenesis (Ait-Belgnaoui et al., 2014; Mohle et al., 2016; Ogbonnaya et al., 2015).

Given the evidence that hippocampal BDNF and adult hippocampal neurogenesis can be regulated by gut microbiota, coupled with the fact that the vagus nerve is a route through which gut microbes can communicate with the brain, it is plausible that vagus nerve activity may in itself be an important modulator of hippocampal plasticity but this has not yet been fully explored. Thus, the aim of this study was to determine whether impairing vagus nerve functioning via subdiaphragmatic vagotomy impacts BDNF mRNA expression and neurogenesis in the adult mouse hippocampus. Since the rodent hippocampus is functionally segregated along its longitudinal axis whereby the dorsal hippocampus (dHi) plays a predominant role in contextual discrimination and spatial learning and memory, whereas the ventral hippocampus (vHi) is predominantly involved in the regulation of anxiety and the stress response (Bannerman et al., 2004; Fanselow and Dong, 2010; Kheirbek et al., 2013; Moser and Moser, 1998; O'Leary and Cryan, 2014; O'Leary et al., 2012; Tanti and Belzung, 2013), we also investigated whether any vagotomy-induced effects on neurogenesis would occur specifically in the dHi or the vHi.

2. Experimental procedures

2.1. Experimental design (see Figure 1)

Mice underwent subdiaphragmatic vagotomy or a sham surgical procedure. One week later, mice received a single injection of cholecystokinin COOH-terminal octapeptide (CCK-8) and its effects on food intake (which is mediated by the vagus nerve) was determined to ensure that the vagotomy and sham surgical procedures were successful. Three days later, mice were injected with BrdU to label proliferating cells. Mice underwent transcardial perfusion 21 days later and using immunohistochemistry the impact of vagotomy on cell proliferation, the survival of 3-week old newly born cells, neurogenesis and neuronal maturation was determined.

2.2. Animals

The tissues used for BDNF measurements were from male BALB/c mice from experiments conducted in McMaster University, Canada, as described previously (Bravo et al., 2011) with approval from the Animal Ethics committee of McMaster University. For the experiments assessing neurogenesis, male BALB/c mice aged 6-7weeks (Harlan, UK) were housed under controlled conditions (temperature 20–21 °C, 55 ± 10% humidity) on a 12 h light/dark cycle and provided with chow and water *ad libitum*. Mice were aged 11–12 weeks at the time of euthanasia. These experiments were conducted in University College Cork in accordance with European Community Council Directive (86/609/EEC) and the Recommendation 2007/526/65/EC, and under a licence provided by the Department of Health and Children.

2.3. Subdiaphragmatic vagotomy

The expression of BDNF was measured in tissue collected from sham and vagotomised animals as previously described (Bravo et al., 2011). For the hippocampal cytogenesis and neurogenesis experiments, subdiaphragmatic vagotomy was conducted as described previously (Bravo et al., 2011). Mice were anaesthetized with isoflurane (5% induction; 2% maintenance). The skin and abdominal wall were incised along the ventral midline, and the intestine was retracted to allow access to the left lateral lobe of the liver, and the stomach. The liver was retracted, and a ligature placed around the gastroesophageal junction to allow gentle pull to clearly visualise the vagal trunks. These were dissected and all neural and connective tissue surrounding the oesophagus below the diaphragm was removed. The sham group underwent a similar operation but the vagus nerve was left intact.

Mice were allowed to recover from surgery for one week prior to assessing whether the vagotomy procedure was successful by measuring cholecystokinin COOH-terminal octapeptide (CCK-8) induced satiety which is mediated by the afferent vagus nerve (Lorenz and Goldman, 1982). Following 20 hours of food deprivation, sham-operated and

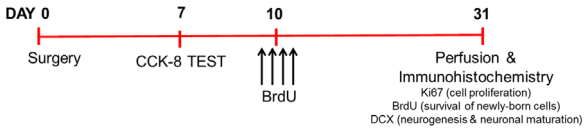

Figure 1 Schematic of experimental design. *Abbreviations:* BrdU, 5-bromo-2'-deoxyuridine; CCK-8; cholecystokinin COOH-terminal octapeptide; DCX, doublecortin.