Neuroscience 267 (2014) 252–262

DIFFERENTIAL ACTIVATION OF THE PREFRONTAL CORTEX AND AMYGDALA FOLLOWING PSYCHOLOGICAL STRESS AND COLORECTAL DISTENSION IN THE MATERNALLY SEPARATED RAT

V. D. FELICE,[a,b] S. M. GIBNEY,[b] R. D. GOSSELIN,[b] T. G. DINAN,[a,c] S. M. O'MAHONY[a,b]* AND J. F. CRYAN[a,b]

[a] *Department of Anatomy and Neuroscience, University College Cork, Ireland*

[b] *Laboratory of Neurogastroenterology, Alimentary Pharmabiotic Centre, University College Cork, Ireland*

[c] *Department of Psychiatry, University College Cork, Ireland*

Abstract—Visceral hypersensitivity is a hallmark of many clinical conditions and remains an ongoing medical challenge. Although the central neural mechanisms that regulate visceral hypersensitivity are incompletely understood, it has been suggested that stress and anxiety often act as initiating or exacerbating factors. Dysfunctional corticolimbic structures have been implicated in disorders of visceral hypersensitivity such as irritable bowel syndrome (IBS). Moreover, the pattern of altered physiological responses to psychological and visceral stressors reported in IBS patients is also observed in the maternally separated (MS) rat model of IBS. However, the relative contribution of various divisions within the cortex to the altered stress responsivity of MS rats remains unknown. The aim of this study was to analyze the cellular activation pattern of the prefrontal cortex and amygdala in response to an acute psychological stressor (open field) and colorectal distension (CRD) using c-fos immunohistochemistry. Several corticoamygdalar structures were analyzed for the presence of c-fos-positive immunoreactivity including the prelimbic cortex, infralimbic cortex, the anterior cingulate cortex (both rostral and caudal) and the amygdala. Our data demonstrate distinct activation patterns within these corticoamygdalar regions including differential activation in basolateral versus central amygdala following exposure to CRD but not the open field stress. The identification of this neuronal activation pattern may provide further insight into the neurochemical pathways through which therapeutic strategies for IBS could be derived.

Key words: maternal separation, corticoamygdalar system, stress, colorectal distension, c-fos, neuronal activation.

INTRODUCTION

A clear etiology and understanding of the mechanisms underlying functional gastrointestinal disorders such as irritable bowel syndrome (IBS) have not been fully elucidated, however dysfunction of the brain–gut axis is thought to be integral to the manifestation of the symptomatology of this disorder (Mulak and Bonaz, 2004; Mayer et al., 2008). Moreover, increased stress susceptibility and co-morbid anxiety and depression are also commonly observed (Gros et al., 2009). Moreover, IBS patients recount a greater amount of early-childhood traumatic events compared with healthy controls (Bradford et al., 2012).

In rodents, neonatal maternal separation (MS) is a well-characterized model of early-life stress, used to model depression, anxiety and IBS (Meaney et al., 1996; Sanchez et al., 2001; Coutinho et al., 2002; Lippmann et al., 2007; Desbonnet et al., 2010; Bravo et al., 2011; O'Mahony et al., 2011; Cotella et al., 2013). When exposed to stressors in adulthood these rats show an altered response to various behavioral paradigms (Gardner et al., 2005; Marais et al., 2008; Lambas-Senas et al., 2009; Troakes and Ingram, 2009; O'Mahony et al., 2009b; Desbonnet et al., 2010). Furthermore, MS evokes an exaggerated increase in blood adrenocorticotropic hormone (ACTH), plasma corticosterone levels, and corticosterone-releasing factor (CRF) in the amygdala, locus coeruleus, and hypothalamus in adulthood (Suchecki et al., 1993, 1995; Plotsky and Meaney, 1993; Barna et al., 2003; Plotsky et al., 2005; O'Malley et al., 2011), all of which have been implicated in depression and IBS. Additionally, these animals also present with increased sensitivity to colorectal distension (CRD) (Coutinho et al., 2002; O'Mahony et al., 2009b), increased gut motility (Schwetz et al., 2005), an altered gut immune response (Barreau et al., 2004), increased intestinal permeability (Soderholm et al., 2002; Gareau et al., 2007), altered fecal microbiota (O'Mahony et al., 2009b), colonic morphology and function (Hyland et al., 2009; O'Malley et al., 2010). These characteristics contribute toward the validation of MS as an ideal model to assess brain–gut axis dysfunction in IBS (O'Mahony et al., 2011).

*Correspondence to: S. M. O'Mahony, Department of Anatomy and Neuroscience, Western Gate Building, Western Road, UCC, Cork, Ireland. Tel: +353-21-420-5479; fax: +353-21-427-3518.
E-mail address: somahony@ucc.ie (S. M. O'Mahony).

Abbreviations: ACC, anterior cingulate cortex; BLA, basolateral nucleus of the amygdala; cACC, caudal ACC; CeA, central nucleus of the amygdala; CRD, colorectal distension; IBS, irritable bowel syndrome; IL, infralimbic cortex; MS, maternally separated; NS, non separated; OF, open field; PBS, phosphate-buffered saline; PrL, prelimbic cortex; PFA, paraformaldehyde; rACC, rostral ACC.

http://dx.doi.org/10.1016/j.neuroscience.2014.01.064
0306-4522/

The role of the prelimbic (PrL) and infralimbic (IL) cortices in anxiety is well documented with previous studies demonstrating that lesions or inactivation of these regions alter anxiety and depression-related behavior in rats compared to controls in various behavioral paradigms (Jinks and McGregor, 1997; Bissiere et al., 2006; Resstel et al., 2008; Slattery et al., 2011). Moreover, activation of these brain regions in response to visceral stimulation has been repeatedly shown by using c-fos immunoreactivity and perfusion mapping (Traub et al., 1996; Wang et al., 2008; Gibney et al., 2010a).

The anterior cingulate cortex (ACC) is also one of the key forebrain regions implicated in anxiety. Alterations in ACC activation have been observed in several human studies on anxiety disorders and depression (Fornito et al., 2008; Etkin et al., 2010, 2011; Shin and Liberzon, 2010), and inactivation of this brain region in rodents has shown anxiolytic and anti-depressogenic effects (Kim et al., 2011). Several studies suggest that the ACC is important in pain perception processing (Sikes and Vogt, 1992; Yamamura et al., 1996; Kwan et al., 2000). Increased cellular activation of the ACC in response to visceral stimulation has been found both in animal models and human studies (Gao et al., 2006a; Larsson et al., 2012; Yan et al., 2012).

The crosstalk between the amygdala and the prefrontal cortex is integral to emotional processing and anxiety (Davidson, 2002). This area receives visceral nociceptive input directly from the spinal cord via the spino-parabrachial–amygdaloid pathway (Menetrey and De Pommery, 1991; Bernard et al., 1996). Furthermore, visceral nociceptive stimulation has previously been demonstrated to increase c-fos immunoreactivity in this brain region (Traub et al., 1996; Nakagawa et al., 2003), whereas inactivation of this region ameliorates the behavioral effects of noxious visceral stimulation (Tanimoto et al., 2003). The immunohistochemical detection of c-fos (an indirect marker of neuronal activity) is a commonly used and well-validated method to analyze brain activation in animal models (Singewald et al., 2003, 2010). In the present study, we also used c-fos expression to compare and contrast open field and CRD-induced cellular activation in the prefrontal cortex and amygdala of the MS rat compared to controls.

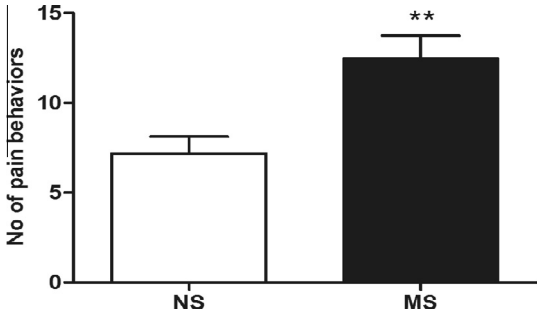

Fig. 2. Graphical representation of the number of muscle contractions over the ten minute interval of the CRD in the rat's abdominal region. This data show an increase in the total number of pain behaviours in MS animals ($^{**}p < 0.01$) indicative of visceral hypersensitivity. Data are expressed as mean ± SEM, ($n = 16$ per group).

EXPERIMENTAL PROCEDURES

Animals

Sprague Dawley rats (250–300 g) were purchased from Harlan (Oxon, UK). Animals were group-housed five per cage and left to habituate for 1 week in the animal facility with food and water *ad libitum*. Sprague-Dawley rat pups were also used in this study. Pups were housed with their mothers in plastic cages (15 × 22 × 9 cm). The animal room remained temperature controlled (20 ± 1 °C) and on 12-h light/dark cycle (lights on at 7:00 AM). The ethics committee of the University College Cork approved the experimental procedure. All experiments were conducted in full accordance with the European Community Council Directive (86/609/EEC). All efforts were made to minimize

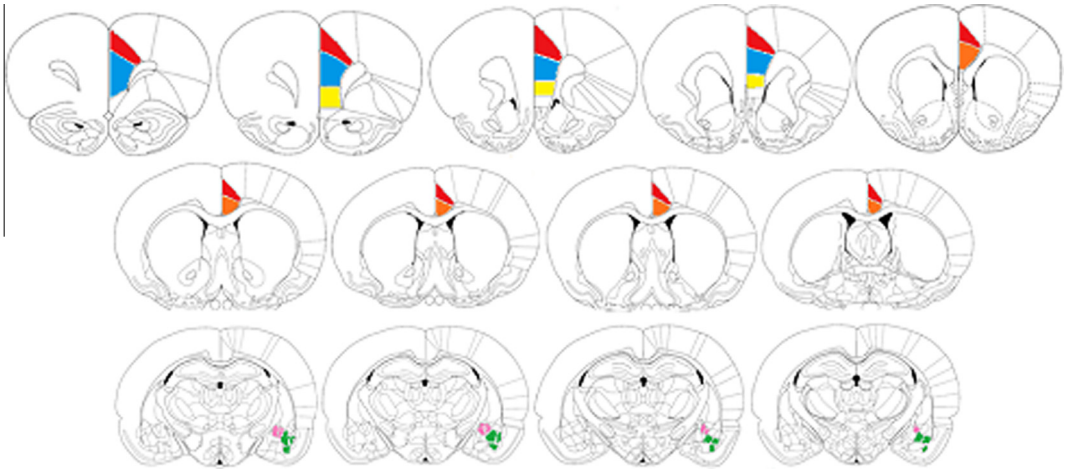

Fig. 1. Schematic diagram showing regions of interest and their anatomical location. Blue, prelimbic cortex (PrL); yellow, infralimbic cortex (IL); red, rostral anterior cingulate cortex (rACC); orange, caudal anterior cingulate cortex (cACC), green central nucleus of the amygdala (CeA), pink, basolateral nucleus of the amygdala (BLA). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)