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Early life stress elicits visceral hyperalgesia and functional reorganization of pain circuits in adult rats

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ABSTRACT

Early life stress (ELS) is a risk factor for developing functional gastrointestinal disorders, and has been proposed to be related to a central amplification of sensory input and resultant visceral hyperalgesia. We sought to characterize ELS-related changes in functional brain responses during acute noxious visceral stimulation. Neonatal rats (males/females) were exposed to limited bedding (ELS) or standard bedding (controls) on postnatal days 2–9. Age 10–11 weeks, animals were implanted with venous cannulas and transmitters for abdominal electromyography (EMG). Cerebral blood flow (rCBF) was mapped during colorectal distension (CRD) using [^{14}C]-iodoantipyrine autoradiography, and analyzed in three-dimensionally reconstructed brains by statistical parametric mapping and functional connectivity. EMG responses to CRD were increased after ELS, with no evidence of a sex difference. ELS rats compared to controls showed a greater significant positive correlation of EMG with amygdalar rCBF. Factorial analysis revealed a significant main effect of 'ELS' on functional activation of nodes within the pain pathway (somatosensory, insular, cingulate and prefrontal cortices, locus coeruleus/lateral parabrachial n. [LC/LPB], periaqueductal gray, sensory thalamus), as well as in the amygdala, hippocampus and hypothalamus. In addition, ELS resulted in an increase in the number of significant functional connections (i.e. degree centrality) between regions within the pain circuit, including the amygdala, LC/LPB, insula, anterior ventral cingulate, posterior cingulate (retrosplenium), and stria terminalis, with decreases noted in the sensory thalamus and the hippocampus. Sex differences in rCBF were less broadly expressed, with significant differences noted at the level of the cortex, amygdala, dorsal hippocampus, raphe, sensory thalamus, and caudate-putamen. ELS showed a sexually dimorphic effect ('Sex x ELS' interaction) at the LC/LPB complex, globus pallidus, hypothalamus, raphe, septum, caudate-putamen and cerebellum. Our results suggest that ELS alters functional

Abbreviations: Acb, Accumbens n; aIns, anterior insula; ANOVA, analysis of variance; cc, corpus callosum; La, lateral amygdala; CA1, CA1 field of hippocampus; Ce, amygdala, central n; Cg1, Cg2, cingulate cortex area 1 dorsal, area 2 ventral; CM, central medial thalamic n; CPu, dorsal caudate-putamen; CON, controls; CRD, colorectal distension; DG, dentate gyrus; dHPC, dorsal hippocampus; Ect/TeA, ectorhinal/temporal association cortex; ELS, early life stress; EMG, electromyography; FC, functional connectivity; GPe, globus pallidus, external; Hb, habenula; HPC, hippocampus; IBS, irritable bowel syndrome; IC, inferior colliculus; La, amygdala, lateral n; LC/LPB, locus coeruleus/lateral parabrachial n; ll, lateral lemniscus; LD, lateral dorsal thalamic n; LO/VO, lateral/ventral orbital cortex; LSI, lateral septum intermediate; M1/M2, primary/secondary motor cortex; MD, mediodorsal thalamic nucleus; Me, amygdala, medial n; MG, medial geniculate; MnR, median raphe n; MPA, hypothalamic medial preoptic area; PAG, periaqueductal gray; PBP, parabrachial pigmented n. of the ventral tegmental area (VTA); pIns, posterior insula; pIns, posterior insula; pIns/Ect, posterior insula/ectorhinal cortex transition area; Po, posterior thalamic n; PrL, prelimbic cortex; PtA, parietal association cortex; PVP, posterior paraventricular thalamic n; rCBF, regional cerebral blood flow; ROI, region of interest; RS, retrosplenial cortex, 'posterior cingulate'; S1BF / S1FL / S1HL / S1J / S1ULp, primary somatosensory cortex, barrel field / forelimb / hindlimb / jaw / upper lip area; S2, secondary somatosensory cortex; SC, superior colliculus; Sim, simple cerebellar lobule; SPM, statistical parametric mapping; STM, bed n. of the stria terminalis; V1/V2, primary/secondary visual cortex; VMH, ventromedial hypothalamus; VMR, visceromotor response; VPL/VPM, ventral-posterolateral/ventral-postereromedial thalamic n.

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activation of the thalamo-cortico-amygdala pathway, as well as the emotional-arousal network (amygdala, locus coeruleus), with evidence that ELS may additionally show sexually dimorphic effects on brain function.

1. Introduction

Increasing evidence suggests that patients with irritable bowel syndrome (IBS) are more likely than healthy subjects to have a history of physical or sexual abuse (Ross, 2005; Walker et al., 1993), trauma (Irwin et al., 1996), and stress (Levy et al., 1997; Son et al., 2008), and in particular early life stress (Bradford et al., 2012; Grad et al., 2014; Halland et al., 2014). In addition, IBS patients compared to control subjects show visceral perceptual alterations during acute (Dickhaus et al., 2003; Elsenbruch et al., 2010; Posserud et al., 2004; Walter et al., 2006) and chronic (Eriksson et al., 2008; Patacchioli et al., 2001) emotional stress. Such findings have prompted hypotheses that life stressors, including those experienced in the early period of life (Chitkara et al., 2008), may trigger long-term changes in brain structure, brain function and visceral sensitivity to noxious stimuli (Chitkara et al., 2008; Murray et al., 2004; Posserud et al., 2004). Based on this, it is believed that part of the symptomatology of patients with functional bowel disorders may be related to a central amplification of sensory input and resultant visceral hyperalgesia, a core symptom of patients with IBS.

Emotions are well known to impact visceral function, and emotional and visceral neuronal circuits are substantially coextensive (Berntson et al., 2003; Bienkowski and Rinaman, 2008; Cameron, 2001; Craig, 2002; King et al., 1999; Price, 1999; Saper, 2002; Thayer and Lane, 2000). A cause and effect relationship between early life stress and visceral hyperalgesia has not yet been established in human subjects. However, prospective studies in preclinical models suggest that raising animals in environments characterized by aberrant maternal care (e.g. neonatal maternal separation (Bian et al., 2010; Coutinho et al., 2002; Welting et al., 2005; Wouters et al., 2012), limited bedding (Guo et al., 2015; Prusator and Greenwood-Van Meerveld, 2015)), results in visceral hyperalgesia in the adult offspring. In rats, central pre-autonomic circuits undergo significant synaptic assembly during the first two weeks of postnatal life (Rinaman et al., 2011). Early life stress (ELS) appears to disrupt maturation of the assembly of limbic, hypothalamic and cortical inputs, to autonomic neurons, and it is hypothesized that this may result in aberrant sensory processing in adult life (Card et al., 2005).

In human subjects, ELS (also referred to as early life or childhood adversity) has recently been associated with altered prefrontal structure, cerebral activity and functional connectivity in the resting state (Cisler et al., 2013; Gupta et al., 2014; Philip et al., 2013b; van der Werff et al., 2013a; van der Werff et al., 2013b), as well as altered limbic/prefrontal brain function during emotion processing (Fonzo et al., 2013). Few studies have investigated the effect of ELS on functional brain activation during noxious visceral stimulation. The current study addresses this in a rodent model of ELS. We also explore sex differences in the effects of ELS on brain functional responses, based on a broad literature demonstrating that women are at greater risk for experiencing many forms of functional pain disorders, including IBS (Berman et al., 2000; Labus et al., 2008; Mayer et al., 2004; Naliboff et al., 2003; Unruh, 1996), as well as a literature suggesting that altered neonatal maternal care may impact adult emotionality and stress responsiveness in a sexually dimorphic manner (Champagne et al., 2008; Champagne and Curley, 2008; Faraday, 2002; Lehmann and Feldon, 2000; Lehmann et al., 1999; Wigger and Neumann, 1999). We hypothesize that ELS-associated visceral hyperalgesia is associated with increased activation in the pain circuit, and that ELS effects on brain functional responses will show sex differences.

2. Materials and methods

2.1. Animals

All experiments were conducted under a protocol approved by the Institutional Animal Care and Use Committee of the University of Southern California and are in accordance with the guidelines of the Committee for Research and Ethical Issues of the International Association or the Study of Pain. Adult female and male Wistar rats (3 month old) were purchased from Harlan Sprague Dawley (Indianapolis, IN, USA) and were housed in the vivarium on a 12-h light/12-h dark cycle with free access to water and rodent chow. Two weeks after arrival to the vivarium, breeding was initiated in separately housed breeding pairs (1 male, 1 female) for the production of male and female offspring. Four litters were used. To minimize potential inter-litter differences in animal behavior, female pups (#2–3) from each litter were randomized to either an ELS/female or control/female group; males were similarly randomized to an ELS/male or control/male group. The following experimental groups were examined: early life stress female (ELS/female, n = 10) and male rats (ELS/male, n = 12); and no-stress female (CON/female, n = 8) and male controls (CON/male, n = 10).

2.2. Limited bedding model

The limited bedding model was introduced by Gilles et al. (Gilles et al., 1996) as an alternate model of maternal neglect and abuse that, unlike the maternal separation model, allows continuous presence of the mother. Minimal nesting material is provided to the dam following delivery of her pups, resulting in increased maternal anxiety and decreased nurturing behavior (licking/grooming), increased rough handling, and increased pup vocalization (Ivy et al., 2008; Raineki et al., 2012). Long-term consequences on the offspring include increased stress hormone release, reduced expression of hypothalamic corticotrophin releasing hormone, adrenal hypertrophy (Avishai-Eliner et al., 2001), decreased social and exploratory behavior (Ivy et al., 2008; Raineki et al., 2012), increased learned helplessness in the Porsolt swim test (Raineki et al., 2012), impaired visual-spatial memory in the Morris Water maze (Ivy et al., 2010), decreased dendritic branching in the CA1 area of the hippocampus (Ivy et al., 2010), increased corticotropin-releasing hormone-positive interneurons in the hippocampus (Ivy et al., 2010), and increased c-fos expression in the amygdala in response to a stress challenge (Raineki et al., 2012). Methods were as previously reported (Guo et al., 2015). One day after testing, plug-positive dams were individually housed under standard vivarium conditions. The day of birth was termed postnatal day 0. On postnatal day 2, litters and dams were transferred to a standard cage equipped with a steel grid (2.5 cm above the floor, 5 × 5 mm mesh). The only bedding material available was a paper towel (1 paper towel for 8–12 pups, ½ towel for <8 pups) that the dam