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SEX DIFFERENCES IN BEHAVIOR AND PRO-INFLAMMATORY CYTOKINE mRNA EXPRESSION FOLLOWING STRESSOR EXPOSURE AND RE-EXPOSURE

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Abstract—Stressful events promote a wide range of neurotransmitter and neuroendocrine changes, which likely serve in an adaptive capacity. However, with repeated stressor exposure, behavioral disturbances, such as anxiety and depression, may develop. Moreover, re-exposure to a stressor for some time following an initial aversive experience may instigate especially pronounced neurochemical variations that favor the emergence of depression and anxiety. These outcomes may stem from any number of neurobiological changes, but increasing attention has focused on the potential contribution of inflammatory factors, such as cytokines. Given the distinct differences in stressor responsiveness that have been reported between males and females, alongside a much higher rate of mood disorders in females, we sought to examine whether repeated exposure to stressors would differentially influence elevated plus-maze behavior in male and female CD-1 mice, and whether such changes would be paralleled by variations of pro-inflammatory mRNA cytokine expression within the prefrontal cortex (PFC) and the hippocampus. In males, the sensitization of interleukin (IL)-1β was evident in both brain regions in those mice that had initially been stressed and then 6 weeks later re-exposed to a stressor exhibiting higher IL-1β expression than did mice stressed on only a single occasion. Females demonstrated higher baseline expression of cytokine expression within the hippocampus, but neither a single exposure nor re-exposure stressor treatment produced significant cytokine elevations. In the PFC an acute stressor treatment increased IL-1R expression, but otherwise had little effect. In a plus-maze test, stressed male mice displayed markedly reduced latencies to the open arms that was evident in a test 6 weeks later irrespective of whether mice were re-exposed to a stressor, whereas in females this outcome was less evident. These studies are consistent with the perspective that female mice are relatively resilient toward stressor-induced cytokine elevations even though in humans females are generally more prone to developing mood disturbances.

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Abbreviations: BDNF, brain-derived neurotrophic factor; C_t, cycle threshold; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL, interleukin; IL-1R, IL-1 receptor; PFC, prefrontal cortex; qPCR, quantitative polymerase chain reaction; TNF-α, tumor necrosis factor alpha.

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INTRODUCTION

Stressful events, through their impact on central neurochemical processes, might enhance an organism's ability to cope successfully with environmental challenges (Anisman et al., 2003). Neurobiological responses to stressors, such as elevated hypothalamic–pituitary–adrenal (HPA) functioning and monoamine activity in frontal cortical regions and the hippocampus are generally viewed as adaptive responses to meet ongoing challenges. However, when stressors occur on a chronic basis, the persistent hormonal or neurochemical changes may result in excessive wear and tear on biological processes that could result in the development of behavioral disturbances, including anxiety and depression (McEwen, 2000).

In addition to the damaging effects of chronic stressors, an organism's stressor history may also affect subsequent responses to stressors. Typically, acute stressors promote neurochemical changes that are fairly transient, persisting for a matter of hours. However, when animals are subsequently re-exposed to stressors, even if they are different from the original challenge, neurochemical changes occur more readily and the magnitude of the changes is more pronounced (Anisman et al., 2003; Belda et al., 2008; Audet et al., 2011). Essentially, the initial stressor session may have engendered the sensitization of processes that are responsible for the elevated response to the later stressor experience (Hayley et al., 2003; Anisman et al., 2008; Morris et al., 2010). In this regard, it has been suggested that these sensitized effects may increase vulnerability to psychological disturbances, such as depression and anxiety (Anisman et al., 2008; Post, 2010) and may contribute to recurring episodes of depression (Espejo et al., 2007; Morris et al., 2010; Post, 2010).

Numerous reports have implicated immune signaling molecules, such as pro-inflammatory cytokines, in the promotion of depressive disorders. In line with this position, stressors increased the mRNA expression and proteins for these cytokines in several brain regions, such as the prefrontal cortex (PFC) and hippocampus, possibly reflecting their release from microglia (Frank et al., 2007). Moreover, sensitization processes may also contribute to stressor-related cytokine variations that occur in the brain (Johnson et al., 2003; Gibb et al.,

2011). The effects observed, however, might be limited to some pro-inflammatory cytokines. For instance, among mice that had been exposed to a social stressor, later exposure to a different social challenge that involved aggressive behavior, markedly increased IL-1β and tumor necrosis factor alpha (TNF-α) mRNA, whereas the expression of IL-6 was reduced (Audet et al., 2011).

Although sex differences occur between males and females in relation to mood disorders (Bebbington, 1998; McCarthy et al., 2005; Karatsoreos and McEwen, 2011), and women are more frequently diagnosed with depression and anxiety disorders (Weich et al., 2001) there are instances in which females are relatively resistant to the detrimental effects of repeated stressors on sensitive brain regions such as the hippocampus (Galea et al., 1997; Conrad et al., 1999; Lin et al., 2008, 2009). Even though cytokines have been implicated in depression, limited data are available concerning sex-specific effects of stressors on cytokine expression. Moreover, data are unavailable regarding potential sex differences of sensitized cytokine changes in the brain. In the current study we examined the immediate effects of a stressor on pro-inflammatory cytokine mRNA expression in male and female mice and assessed whether sex-specific cytokine changes would be elicited on the basis of an earlier stressor experience.

EXPERIMENTAL PROCEDURES

Animals

Male and female CD-1 mice, purchased from Charles River Canada, St. Constant, Quebec, were used as experimental subjects. Upon arrival, mice were approximately 45 days old and were allowed 2 weeks to become acclimatized to the laboratory. They were housed individually in standard-sized (27 × 21 × 14 cm) polypropylene cages and were exposed to 12-h light–dark cycles (from 07:00 to 19:00 h), with the temperature and humidity kept constant (22 °C and 63%, respectively). The animals had free access to food and water. The study met the guidelines laid out by the Canadian Council on Animal Care and was approved by the Carleton University Animal Care Committee.

Procedure

Mice that had been individually housed were exposed (or not) to a variable stressor regimen for 3 consecutive days (days 70–72). The mice were then not disturbed for 6 weeks. At this point mice were randomly assigned to either restraint stress or no treatment (N = 8–10 per group). Following the stress exposure, mice were returned to their cages for 90 min and were sacrificed by rapid decapitation or exposed to the elevated plus-maze. In the rapid decapitation, brains were quickly removed and placed on a stainless steel brain matrix (2.5 × 3.75 × 2.0 cm), which was located on a block of ice. The brain matrix was made up of a series of stainless steel plates that had a series of slots spaced 500 μm apart that guided razor blades to provide coronal brain sections. Once the brains were sliced, tissue punches from the PFC and hippocampus were collected by micropunch using hollow 20-gauge needles with a beveled tip following the mouse atlas of Franklin and Paxinos (1997). Tissue punches were stored at −80 °C for subsequent determination of cytokine mRNA expression. This included analyses of mRNA expression of IL-1β, IL-6, TNF-α and their respective receptors, IL-1 receptor (IL-1R), IL-6 receptor (IL-6R)and TNF-α (TNF-αR) receptor in the PFC and the hippocampus, brain regions associated with cytokine activation and stress regulation (Anisman et al., 2003; Hayley et al., 2003).

Initial variable stressor

The mice were initially exposed to a 3-day variable stressor regimen adapted from Jacobson-Pick and Richter-Levin (2010) or not exposed to any stressor. The variable stressor regimen consisted of (a) 10-min physical tight restraint in a snug plastic triangular bag (with a hole cut at the snout) (Day 1), (b) 10-min forced swim in water of 21 °C within a 30-cm (diameter) × 43-cm (height) cylinder (Day 2), and (c) placement in a cage containing water-soaked bedding for 1 h (Day 3). After the 3-day stressor regimen, mice were returned to their home cages and were not disturbed for 6 weeks (except for routine cage maintenance). All stressors were applied between 08:30 and 13:00 h to minimize behavioral effects related to diurnal factors. The non-stressed mice remained in their cages undisturbed during the 3-day period.

Acute stressor

Randomly assigned mice were subject to an acute restraint stressor, which ordinarily influences monoamine systems within limbic brain regions (Buynitsky and Mostofsky, 2009) or were not stressed (resulting in a control group that experienced neither the initial or test day acute stressors). The acute stressor comprised of a 15-min physical restraint in a snug plastic triangular bag (with a hole in the tip of the bag for the mouse's snout) providing tight restraint. This stressor has been found to influence various neurotransmitters in our previous research (Gibb et al., 2008; Jacobson-Pick et al., 2012). Animals that were not exposed to the restraint stressors were brought to the laboratory and placed in a room separate from where the other mice were being stressed.

Reverse transcription-quantitative polymerase chain reaction analysis

Brain tissue punches were homogenized using Trizol and total brain RNA was isolated according to the manufacturer's instructions (Invitrogen, Burlington, ON, Canada). The total RNA was then reverse-transcribed using Superscript II reverse transcriptase (Invitrogen, Burlington, ON, Canada) and the resulting cDNA aliquots were analyzed in simultaneous quantitative polymerase chain reactions (qPCR). SYBR green detection was used according to the manufacturer's