Progress in Neuro-Psychopharmacology & Biological Psychiatry 33 (2009) 251–261

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnpbp

Sex differences in corticolimbic dopamine and serotonin systems in the rat and the effect of postnatal handling

Annie Duchesne, Marc M. Dufresne, Ron M. Sullivan *

Centre de Recherche Fernand-Seguin, Département de Psychiatrie, Université de Montréal, Canada

ARTICLE INFO

Article history:
Received 25 June 2008
Received in revised form 19 November 2008
Accepted 20 November 2008
Available online 3 December 2008

Keywords:
Dopamine
Gender
Handling
Lateralization
Serotonin
Stress

ABSTRACT

Stress-related psychopathology is particularly prevalent in women, although the neurobiological reason(s) for this are unclear. Dopamine (DA) and serotonin (5-HT) systems however, are known to play important adaptive roles in stress and emotion regulation. The aims of the present study included examination of sex differences in stress-related behaviour and neuroendocrine function as well as *post mortem* neurochemistry, with the main hypothesis that corticolimbic DA and 5-HT systems would show greater functional activity in males than females. Long–Evans rats of both sexes were employed. Additional factors incorporated included differential postnatal experience (handled vs. nonhandled) and adult mild stress experience (acute vs. repeated (5) restraint). Regional neurochemistry measures were conducted separately for left and right hemispheres. Behaviourally, females showed more exploratory behaviour than males in the elevated plus maze and an openfield/holeboard apparatus. Females also exhibited significantly higher levels of adrenocorticotrophic hormone and corticosterone at all time points in response to restraint stress than males across treatment conditions, although both sexes showed similar habituation in stress-induced ACTH activation with repeated mild stress. Neurochemically, females had significantly higher levels of DA (in ventromedial prefrontal cortex (vmPFC), insular cortex and n. accumbens) and 5-HT (in vmPFC, amygdala, dorsal hippocampus and insula) than males. In contrast, males had higher levels of the DA metabolite DOPAC or DOPAC/DA ratios than females in all five regions and higher levels of the 5-HT metabolite 5-HIAA or 5-HIAA/5-HT ratios in vmPFC, amygdala and insula, suggesting greater neurotransmitter utilization in males. Moreover, handling treatment induced a significant male-specific upregulation of 5-HT metabolism in all regions except n. accumbens. Given the adaptive role of 5-HT and DAergic neurotransmission in stress and emotion regulation, the intrinsic sex differences we report in the functional status of these systems across conditions, may be highly relevant to the differential vulnerability to disorders of stress and emotion regulation.

1. Introduction

The incidence of stress-related psychiatric conditions like clinical depression, anxiety disorders and post-traumatic stress disorder, is approximately doubled in females compared to males (Kessler, 2003; Holden, 2005). Large scale studies have concluded that this gender discrepancy is not accounted for by a wide range of socio-demographic variables or by the number or type of social roles occupied (Weich et al., 1998; Klose and Jacobi, 2004). What is certain, is that the male and female brain (be it human or rodent) differs generally on many levels, including anatomically, metabolically and neurochemically (Witelson, 1991; Witelson et al., 1995; Murphy and Hepworth, 1996; Biver et al., 1996; Kaasinen et al., 2002; Wager et al., 2003; Hall et al., 2004; Cahill, 2006, 2005), and specifically in the activational responses to emotional stimuli (Killgore and Yurgelun-Todd, 2001, 2004; Canli et al., 2002; Kemp et al., 2004; White et al., 2005; Cahill, 2005; Tranel et al., 2005).

The hypothalamic-pituitary-adrenal (HPA) axis plays a central role in responding to and coping with stress. Clinical studies have shown HPA axis function to be sexually dimorphic in both normal and pathologic conditions such as major depressive disorder, as females have higher cortisol levels and are more resistant to HPA axis suppression with dexamethasone (Young, 1998; Klein and Corwin, 2002; Kudielka and Kirschbaum, 2005). In rats, sex differences have been demonstrated in HPA axis function and in the behavioural

Abbreviations: Ac, Acute (restraint) stress; ACTH, Adrenocorticotrophic hormone; CORT, Corticosterone; DA, Dopamine; DHBA, Dihydroxybenzylamine; DOPAC, 3,4-dihydroxyphenylacetic acid; EPM, Elevated plus maze; F, Female; H, Handling or Handled; HPA, Hypothalamic-pituitary-adrenal; HPLC-EC, High performance liquid chromatography-electrochemical detection; L, Left; M, Male; mRNA, Messenger ribonucleic acid; NE, Norepinephrine; NH, Nonhandling or Nonhandled; Rp, Repeated (restraint) stress; R, Right; vmPFC, Ventromedial prefrontal cortex; 5-HT, 5-hydroxytryptamine (serotonin); 5-HIAA, 5-hydroxyindole acetic acid.

* Corresponding author. Centre de Recherche Fernand-Seguin, 7331 rue Hochelaga, Montréal, Québec, Canada H1N 3V2. Tel.: +1 514 251 4015; fax: +1 514 251 2617.
E-mail address: ron.sullivan@umontreal.ca (R.M. Sullivan).

0278-5846/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.pnpbp.2008.11.012

responses to stressful or anxiety-provoking situations (Blanchard et al., 1991; Bowman et al., 2003; Karandrea et al., 2000, 2002; Beiko et al., 2004; Curtis et al., 2006). Relative to males, females have higher levels of the plasma stress hormones adrenocorticotrophic hormone (ACTH) and corticosterone, and tend to show more exploratory behaviour (Johnston and File, 1991; Mendelson and McEwen, 1991; Viau and Meaney, 1991; Imhof et al., 1993; Burgess and Handa, 1992; Lunga and Herbert, 2004). It should be noted however, that the nature of sexual dimorphism on HPA axis measures is more complex in humans.

Central monoamine activity in limbic and cortical terminal regions also plays an integral role in the coordination of behavioural and physiological responses to stress, and in the adaptive maintenance of homeostasis. Moreover, the majority of pharmacotherapies in the treatment of stress-related psychopathology enhance serotonin (5-HT) neurotransmission specifically, and frequently norepinephrine (NE) and dopamine (DA) function as well. However, studies have suggested that these systems can exhibit sex differences in their functional nature and in response to various challenges (Carlsson et al., 1985; Becker, 1999; Andersen and Teicher, 2000; Dluzen and McDermott, 2000).

Early life adversity is known to be a predisposing factor in the development of many stress-related psychopathologies like depression (Graham et al., 1999; Heim et al., 2004), and early life events can greatly influence the long-term programming of neuroendocrine systems and behaviour. For example, postnatal handling (H) stimulation greatly modifies the development of central systems involved in stress and emotion regulation (Levine et al., 1957; Meaney et al., 1991). However, the long-term effects of early H treatments on stress regulatory systems may also be sex-specific (Papaioannou et al., 2002; Park et al., 2003; Panagiotaropoulos et al., 2004; Severino et al., 2004).

The purpose of the present study was to examine the nature of sex differences on a variety of behavioural, neuroendocrine and neurochemical endpoints, by incorporating a number of potentially important modulating factors in the same study design. Males and females from both H and nonhandled (NH) treatments were studied. We also incorporated the effect of differential (acute vs. repeated) mild stress experience on neuroendocrine and neurochemical endpoints. The latter assessment included five key brain regions in stress and emotion regulation seldom examined in the same study, and also incorporated the factor of hemisphere, as cerebral laterality can be a significant factor in stress/emotion processing (Denenberg, 1981; Sullivan, 2004; Sullivan and Dufresne, 2006; Sullivan and Szechtman, 1995). Our aims included the confirmation and extension of sex differences in behaviour and neuroendocrine function. We additionally hypothesized that males would show more pronounced neuroendocrine habituation to repeated mild stress than females, as has been previously noted in certain stress paradigms (Galea et al., 1997). We predicted that males would show a generally greater degree of monoamine utilization in these brain regions than females, and that any "adaptive" effects of H treatment would be more pronounced in males than females.

2. Methods

All procedures in the present study conformed to the guidelines of the Canadian Council on Animal Care, with approval from the local hospital (Louis-H. Lafontaine) research ethics committee, while attempting to employ the minimum number of animals possible.

2.1. Animals and neonatal handling procedure

Pregnant (12–13 day gestation) Long–Evans rats (Charles River, St. Constant, Québec, Canada) were acclimated to the animal facility for 8–10 days before giving birth, and maintained on a 12-h light/dark cycle with free access to food and water.

Following birth, litters were culled to a maximum of 12 pups per litter with roughly equal numbers of males and females. Litters contained a minimum of 4 pups of each sex and a maximum of 6 pups of each sex. Whole litters were randomly assigned to either H or NH treatments, with 4 litters per treatment. Neonatal H was performed daily for 14 days beginning the day after birth, between 10:00 and 11:00 h as follows. First, the dams were removed to an adjacent cage lined with regular bedding. Pups were then removed from the homecage with the entire litter placed in a cage lined with paper towel for 15 min. Pups were then returned to the homecage, followed by the return of the dam. During this 14 day period, NH litters were left undisturbed except for cage maintenance conducted once on day 7. Standard (biweekly) cage maintenance was resumed on day 15 and all animals were weaned on day 21 and pair housed with rats of the same sex and treatment condition until adulthood.

2.2. Elevated plus maze

Between 60 and 70 days of age, rats were videotaped for 5 min in the elevated plus maze (EPM) to measure anxiety-like behaviour (Handley and McBlane, 1993; Pellow et al., 1985). The test was performed between 10:00 and 14:00 h under low light conditions to encourage exploration. Each rat was placed in the center square of the maze (10×10 cm) facing an open arm. Arms of the maze were 45×10 cm. The walls of the closed arms were 45 cm high and the open arms were bordered by a 0.5 cm high lip to prevent the rats from falling off (60 cm above the floor). The primary measures of interest were the number of entries and time spent in the open and closed arms, ratio of time in open/closed arms, as well as defensive stretch/attend behaviours. Entries were defined as having all four paws entering an arm of the maze.

2.3. Holeboard testing

A week following plus maze testing, rat behaviour was videotaped for 10 min in a holeboard/openfield apparatus (100×100×50 cm) with a raised floor containing 9 equidistant holes (5 cm diameter). The total number of holes (1 center and 8 outer) actively investigated was recorded. The purpose of this test was twofold. First, this served as a behavioural indicator of the effectiveness of the H procedure, as we typically observe that H rats investigate significantly (approximately 30%) more holes than NH rats (e.g. Sullivan and Dufresne, 2006). Second, this provided a measure of individual behavioural differences by which rats could be ranked and assigned to the subgroups described below, assuring that subgroups for stress testing were equivalent from a behavioural/activity baseline and that litter effects could be minimized for neuroendocrine and neurochemical measures.

2.4. Acute and repeated restraint stress

Rats within individual (H or NH) litters were ranked from high to low on the basis of total holes explored in the above test. Then rats of each sex within litters were alternately assigned to one of two subgroups receiving either acute (Ac) restraint or repeated (Rp) restraint testing. For example, if the highest ranking female from a given litter was assigned to the Ac subgroup, the highest ranking female from the next litter would be assigned to the Rp subgroup, and so on. Thus, rats from each of the 4 main conditions (H-males, H-females, NH-males, NH-females), were split into Ac or Rp stress subgroups. Moreover, each of the 4 subgroups within each H or NH treatment, contained similar numbers of rats from each original litter to control for litter effects. This resulted in a 2×2×2 study design with an n=9–12 for each of the eight subgroups.

Beginning the week following holeboard testing, rats in the acute condition were placed in plexiglas restrainers at room temperature for 20 min between 10:00 and 13:00 h. Blood samples were taken from the tail vein at 0, 20 and 80 min corresponding to pre-stress, peak stress and stress recovery time points. The first sample was taken