Int. J. Devl Neuroscience 44 (2015) 33–47

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

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

Early-life stress increases the survival of midbrain neurons during postnatal development and enhances reward-related and anxiolytic-like behaviors in a sex-dependent fashion

Agnieszka Chocyk*, Iwona Majcher-Maślanka, Aleksandra Przyborowska, Marzena Maćkowiak, Krzysztof Wędzony

Laboratory of Pharmacology and Brain Biostructure, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Kraków, Poland

ARTICLE INFO

Article history:
Received 4 February 2015
Received in revised form 7 May 2015
Accepted 8 May 2015
Available online 14 May 2015

Keywords:
Anxiety
Early-life stress
Substantia nigra
Sucrose preference
Ventral tegmental area

ABSTRACT

Clinical studies have suggested that early-life stress (ELS) increases the risk of psychopathologies that are strongly associated with dysfunction of dopaminergic neurotransmission. Thus, ELS may interfere with the development and maturation of the dopaminergic system; however, the mechanisms involved in such interference are poorly understood. In the present study, we investigated the effect of ELS on the survival of specific populations of neurons in the substantia nigra *pars compacta* (SNc) and ventral tegmental area (VTA) during postnatal development. First, we injected bromodeoxyuridine (BrdU) into pregnant rat dams on embryonic days 12, 13 and 14 to permanently label midbrain neurons. Then, after birth, the dams and litters were subjected to a maternal separation (MS) procedure to model ELS conditions. The number of BrdU+ neurons and the total number of neurons (cresyl violet+, CV+) were estimated in both male and female juvenile, adolescent, and adult rats. Moreover, sucrose preference and anxiety-like behaviors were studied during adulthood. We found that MS permanently increased the number of BrdU+ and CV+ neurons in the VTA of males. In the SNc, a temporary increase in the number of BrdU+ neurons was observed in juvenile MS males; however, only adult MS males displayed an increase in the number of CV+ neurons. Immunofluorescence analysis implied that MS affected the fate of non-dopaminergic neurons. MS males displayed anxiolytic-like behavior and an increase in sucrose preference. These results suggest that ELS induces distinct dysregulation in the midbrain circuitry of males, which may lead to sex-specific psychopathology of the reward system.

1. Introduction

During the last decade, numerous clinical and epidemiological studies have convincingly revealed that early-life stress (ELS) may increase the risk for later psychopathologies, such as mood and anxiety disorders, behavior disorders and substance use disorders (Andersen and Teicher, 2008, 2009; Green et al., 2010; Kessler et al., 2010). Disturbances in the neural circuits associated with dopaminergic neurotransmission have been strongly implicated in the pathophysiology of the disorders mentioned above (Schultz, 2007; Van den Heuvel and Pasterkamp, 2008). Interestingly, many functional neuroimaging studies in the human brain have shown correlations between ELS and dopaminergic system dysfunction and have suggested that ELS interferes with brain development and/or maturation, thus leading to dopaminergic circuitry dysfunction and later psychopathology (Pruessner et al., 2004; Dillon et al., 2009; Oswald et al., 2014).

To investigate the effects of ELS on brain development and maturation, several animal models have been developed, including (1) prenatal stress (Kippin et al., 2008; Hausknecht et al., 2013), (2) perinatal glucocorticoid (GC) exposure (McArthur et al., 2007; Virdee et al., 2014), and (3) maternal (or parental) separation (Brake et al., 2004; Chocyk et al., 2010, 2011a,b; Braun et al., 2013). Behavioral and biochemical data have strongly indicated that animals subjected to ELS procedures display sex-dependent functional changes in the dopaminergic system (Hall et al., 1999; Kosten et al., 2005; Chocyk et al., 2011b; Kunzler et al., 2015; Virdee et al., 2014). In general, prenatal stress and maternal separation (MS) increase behavioral responses to psychostimulants (Brake et al., 2004; Kikusui et al., 2005; Kosten et al., 2005; Kippin et al., 2008; Chocyk et al., 2011b; Hausknecht et al., 2013). At the biochemical level, much more sexually dimorphic responses to ELS have

* Corresponding author. Tel.: +48 12 6623 229; fax: +48 12 637 45 00.
E-mail address: chocyk@if-pan.krakow.pl (A. Chocyk).

http://dx.doi.org/10.1016/j.ijdevneu.2015.05.002
0736-5748/

been observed. ELS increases cocaine- or amphetamine-induced dopamine release in the nucleus accumbens, striatum and medial prefrontal cortex (mPFC) principally in males (Hall et al., 1999; Kosten et al., 2005; Kippin et al., 2008; Virdee et al., 2014). The opposite effect, i.e., a decrease in amphetamine-induced mesolimbic dopamine release, has been observed in female offspring subjected to antenatal GC treatment (Virdee et al., 2014).

Many neuropsychiatric and neurological disorders are characterized by substantial sex differences in their susceptibility and prevalence (Ngun et al., 2011; Bao and Swaab, 2011). For instance, depression and anxiety disorders are more frequent in women, whereas in men, substance abuse, schizophrenia and Parkinson's disease are more prevalent (Ngun et al., 2011; Bao and Swaab, 2011). These clinical facts suggest sexual differentiation of the brain, particularly in the dopaminergic system. Both animal and human studies have confirmed this hypothesis (Ngun et al., 2011; Bao and Swaab, 2011; Gillies et al., 2014). These sex-related factors may interact with the early-life environment (e.g., ELS) and further influence the vulnerability and resilience of individuals to mental and neurological disorders.

The mechanisms underlying ELS-induced functional abnormalities in dopaminergic neurotransmission are poorly understood. Developmental changes in the number and/or function of dopaminergic neurons located in the substantia nigra *pars compacta* (SNc) and ventral tegmental area (VTA) have been hypothesized to have a potential impact on the behavioral and biochemical phenotypes of animals and humans subjected to ELS. Classically, the SNc and VTA were considered dopaminergic structures; however, recently accumulated data have shown that these nuclei are far more heterogeneous (Yamaguchi et al., 2007; Nair-Roberts et al., 2008; Margolis et al., 2012; Yamaguchi et al., 2013). Currently, in addition to a prominent population of dopaminergic tyrosine hydroxylase-positive (TH^+) neurons (~60%), TH-negative purely GABAergic (~30%) and glutamatergic neurons (~2–5%) are known to be present in the VTA and SNc (Yamaguchi et al., 2007, 2013; Nair-Roberts et al., 2008; Margolis et al., 2012; Walsh and Han, 2014). GABAergic and glutamatergic neurons in the midbrain belong to locally acting interneurons and to projection neurons (Dobi et al., 2010; Gorelova et al., 2012; van Zessen et al., 2012; Brown et al., 2012; Taylor et al., 2014). Moreover, data demonstrating the role of non-dopaminergic (GABAergic and glutamatergic) midbrain neurons in the regulation of the reward circuit have rapidly accumulated (Dobi et al., 2010; van Zessen et al., 2012; Brown et al., 2012).

Knowledge regarding the effects of ELS on the specific populations of VTA and SNc neurons is scarce. A few groups, including our group, have studied the effect of ELS on the number of dopaminergic neurons in the SNc and VTA using immunohistochemical detection of TH (Braun et al., 2000; McArthur et al., 2007; Chocyk et al., 2011b). TH is a rate-limiting enzyme in dopamine synthesis and is a commonly used marker of dopaminergic cell bodies in the midbrain (Bjorklund and Dunnett, 2007). Perinatal GC exposure increases the number of TH-immunoreactive (IR) neurons in the SNc and VTA of adult males and females (McArthur et al., 2007). Our previous study revealed a similar effect on the VTA of adult females subjected to the MS paradigm (Chocyk et al., 2011b). However, we also observed that MS transiently decreased the number of TH-IR cells in the VTA of both male and female juvenile rats (Chocyk et al., 2011b). Simultaneously, we detected age-dependent (developmental) increases in the number of TH-IR neurons in control and MS rats, particularly in males (Chocyk et al., 2011b). Considering that (1) all dopaminergic neurons are generated prenatally (Zecevic and Verney, 1995; Orme et al., 2009), (2) postnatal neurogenesis in the midbrain remains controversial (Frielingsdorf et al., 2004; Shan et al., 2006; Zhao and Janson Lang, 2009), and (3) gonadal hormones (testosterone and estradiol) are known to affect the enzymatic and transcriptional activity of TH (Kumai et al., 1995; Jeong et al., 2006; Sabban et al., 2010; Maharjan et al., 2010), our study and the studies of others have revealed the effects of ELS, age and sex on TH expression (as protein) in particular neurons but not on the number of dopaminergic neurons *per se*. Therefore, TH immunodetection has a serious limitation when applied to the examination of developmental changes in the entire population of dopaminergic neurons. This methodological problem also affects protein markers of non-dopaminergic neurons (e.g., glutamic acid decarboxylase, GAD67 or the vesicular glutamate transporter, VGLUT) because we cannot rule out the possibility that stress, age and sex affect the synthesis of these protein markers and subsequently affect the observed cell phenotype.

The methodological limitations discussed above inspired us to look for an alternative paradigm that would enable us to trace the effect of ELS on the number of VTA and SNc neurons during postnatal development. In the present study, we injected bromodeoxyuridine (BrdU), which is a synthetic analog of thymidine, into pregnant rat dams during the period of intensive midbrain neurogenesis (on embryonic days (E) 11, E12 and E14 (Gates et al., 2006; Orme et al., 2009)) to permanently label specific populations of VTA and SNc neurons. BrdU integrates into DNA of dividing cells during DNA synthesis in S phase; therefore, BrdU is a stable marker of cells born around the time of injection (Gates et al., 2006; Magavi and Macklis, 2008; Orme et al., 2009). After birth, from postnatal day (PND) 1 to PND 14, the dams and litters were subjected to a MS procedure, and then BrdU-labeled neurons were detected using immunohistochemistry in both male and female juvenile, adolescent and adult animals. The purpose of this study was to establish the effects of MS on the survival of BrdU-labeled neurons and on the total number of neurons in the VTA and SNc over the lifespan. The phenotype of BrdU+ cells was also studied using double-label immunofluorescence analysis. Additionally, the behavioral phenotype of adult animals was of potential interest. Recent data have suggested that midbrain circuitry regulates not only responses to reward-related and aversive stimuli but also the expression of anxiety and depressive-like symptoms (Tsai et al., 2009; Tan et al., 2012; Jennings et al., 2013; Tye et al., 2013). Therefore, in this study, we measured natural reward consumption using the sucrose preference test and anxiety-like behaviors using the light/dark exploration test.

2. Materials and methods

2.1. Animals

All experimental procedures were approved by the Committee for Laboratory Animal Welfare and the Ethics Committee of the Institute of Pharmacology, PAS, in Krakow and met the requirements of the European Council Guide for the Care and Use of Laboratory Animals (86/609/EEC).

Primiparous Wistar dams (Charles River, Germany) were mated in the animal facility at the Institute of Pharmacology, PAS, in Krakow. The dams were individually housed under standard conditions with an artificial 12 h light/dark cycle (lights on from 07:00 to 19:00 h), and food and tap water were freely available. Pregnant dams were injected with 5-bromo-2′-deoxyuridine (BrdU, 30 mg/kg/day ip, Sigma, USA) on consecutive embryonic days E12, E13 and E14 (i.e., single BrdU injection every 24 h for three days). Some pregnant females were left undisturbed (no injection was implemented). The date of birth was designated as postnatal day (PND) 0. On PND 1, the pups and dams were assigned randomly to one of the following rearing conditions: maternal separation (MS) or animal facility rearing (AFR). Additionally, the litter size was standardized to eight pups per litter (four males and four females).