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The effects of early-life stress on dopamine system function in adolescent female rats

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ABSTRACT

During adolescence, many neural systems, including the dopamine system, undergo essential remodeling and maturation. It is well known that early-life stress (ELS) increases the risk for many psychopathologies during adolescence and adulthood. It is hypothesized that ELS interferes with the maturation of the dopamine system. There is a sex bias in the prevalence of stress-related mental disorders. Information regarding the effects of ELS on brain functioning in females is very limited. In the current study, maternal separation (MS) procedures were carried out to study the effects of ELS on dopamine system functioning in adolescent female rats. Our study showed that MS increased the density of tyrosine hydroxylase immunoreactive fibers in the prelimbic cortex (PLC) and nucleus accumbens (Acb). These changes were accompanied by a decrease in the level of D5 receptor mRNA and an increase in D2 receptor mRNA expression in the PLC of MS females. Conversely, D1 and D5 receptor mRNA levels were augmented in the caudate putamen (CPu), while the expression of the D3 dopamine receptor transcript was reduced in MS females. Additionally, in the Acb, MS elicited a decrease in D2 receptor mRNA expression. At the behavioral level, MS increased apomorphine-induced locomotion; however, it did not change locomotor responses to selective D1/D5 receptor agonist and attenuated D2/D3 receptor agonist-triggered locomotion. Moreover, MS decreased D1/D5 receptor agonist-induced grooming behavior. These results indicate that ELS disrupts dopamine receptor function in the PLC and basal ganglia during adolescence in females and may predispose them to psychopathologies during adolescence and adulthood.

1. Introduction

Adolescence is a time of the gradual developmental transition between childhood and adulthood. It is recognized not only in humans but also in nonhuman primates and rodents. (Spear, 2000). Adolescence is characterized by specific behavioral changes, i.e., an increase in peer-directed social interactions, increased risk-taking, recklessness and novelty-seeking behaviors (Spear, 2000; Sturman and Moghaddam, 2011). These behaviors have been implicated in the prevalence of drug and alcohol use during adolescence in humans (Spear, 2000; Sturman and Moghaddam, 2011). An accumulation of evidence indicates that many regions of the adolescent brain undergo a process of maturation and significant remodeling (Spear, 2000; Sturman and Moghaddam, 2011). It has been suggested that such developmental changes within the dopamine system may underlie specific behaviors and pathophysiology of mental disorders in adolescents. Dopamine neurotransmission is involved in many essential processes. Specifically, the nigrostriatal dopamine pathway, which originates in the substantia nigra (SN) and projects to the dorsal striatum, i.e., the caudate putamen (CPu), regulates motor functions and goal-oriented behaviors. The mesolimbic pathway sends projections from the ventral tegmental area (VTA) to the ventral striatum, i.e., the nucleus accumbens (Acb), and is engaged in motivation and reward processes. The mesocortical dopamine pathway connects the VTA with the prefrontal cortex (PFC) and regulates cognition and stress responses (Bjorklund and Dunnett, 2007; Schultz, 2007). It has been recognized that the number of dopamine terminals in the PFC increases during adolescence (Kalsbeek et al., 1988; Rosenberg and Lewis, 1994). At the same time, a massive loss of overproduced synapses in the PFC and striatum has been observed in humans, nonhuman primates and rodents (Spear, 2000; Sturman and Moghaddam, 2011). Moreover, the overproduction of D1 and D2 dopamine receptors and subsequent pruning has also been shown to occur in the PFC and striatum (Andersen et al., 1997, 2000; Brenhouse et al., 2008;

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Montague et al., 1999). However, it is worth noting that what is known of dopamine system maturation and functioning during ontogenesis is primarily based on studies in male subjects (Brenhouse et al., 2008; Chen et al., 2010; Dwyer and Leslie, 2016). Some studies have shown that the overproduction and pruning of dopamine receptors and synapses are less pronounced in females (Andersen et al., 1997, 2002).

The developing brain is very sensitive to stressful insults during adolescence due to ongoing brain remodeling processes (McCormick and Mathews, 2010). Moreover, the consequences of these perturbations may easily emerge during adolescence. Therefore, it is not surprising that many different psychopathologies, such as attention deficit hyperactivity disorder (ADHD), schizophrenia, anxiety, depression, conduct disorder and substance use disorder, emerge during adolescence (Andersen and Teicher, 2008; Andersen and Teicher, 2009; Zahn-Waxler et al., 2008). Interestingly, there is a sex bias in the prevalence of some mental disorders among adolescents. For instance, anxiety, depression and eating disorders are more common in adolescent females, while adolescent males have a greater prevalence of ADHD, schizophrenia, conduct disorders and substance abuse (Gillies and McArthur, 2010; Zahn-Waxler et al., 2008). It is generally accepted that early-life stress (ELS) is a risk factor for developing metal disorders in all stages of life (Kessler et al., 2010). Numerous neuroimaging studies in humans have demonstrated that ELS impacts dopamine system functioning (Dichter et al., 2012; Dillon et al., 2009). Therefore, it has been hypothesized that ELS interferes with the maturation of the dopamine system. Many biochemical and behavioral studies have indicated that ELS affects dopamine neurotransmission in adult animals (Brake et al., 2004; Kosten et al., 2005; Matthews et al., 1999; McArthur et al., 2007; Ploj et al., 2003). Our own previous studies revealed that ELS induced by maternal separation (MS) enhanced responses to acute cocaine injection during a locomotor activity test in adult male and female rats (Chocyk et al., 2011). However, relatively little is known regarding the manifestations of ELS-induced abnormalities in the dopamine system during adolescence, particularly in female subjects (Brenhouse et al., 2013; Chocyk et al., 2011, 2015; Jahng et al., 2010; Li et al., 2013). We reported previously that MS increased the number of tyrosine hydroxylase-immunoreactive (TH-IR) dopamine neurons, particularly in the SN *pars reticulata* (SNr) of adolescent females (Chocyk et al., 2011). Together, these findings prompted us to further investigate whether ELS, modeled by the MS procedure, affects the dopamine system in adolescent female rats. Specifically, we investigated the effects of MS on the density of TH-IR terminals and D1-like and D2-like dopamine receptor mRNA levels in key brain regions of the dopamine pathway, i.e., the prelimbic cortex (PLC), which is a part of the medial PFC, and the CPu and Acb. At the behavioral level, we investigated the locomotor activity of rats in response to novelty and the injection of dopamine receptor agonists including apomorphine (nonselective dopamine receptor agonist), SKF 77434 (D1-like receptor agonist) and quinpirole (D2-like receptor agonist). Additionally, we examined the impact of MS on grooming behavior induced by SKF 77434.

2. Material 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).

The offspring of primiparous Wistar dams (Charles River, Germany) mated at the Institute of Pharmacology, PAS, Krakow Animal Facility were used in this study. Before delivery, the dams were housed individually in standard plastic cages (Type III H, 38 × 24 × 19 cm) under controlled conditions with an artificial 12 h light/dark cycle (lights on from 07.00 to 19.00). Food and tap water were freely available. The day of birth was designated as postnatal day (PND) 0. On PND 1, the litter size was standardized to eight pups per litter (four males and four females), and the litters were assigned to one of the following rearing conditions: maternal separation (MS) or animal facility-rearing (AFR). Animals remained in the assigned rearing conditions until PND 14.

2.1.1. Maternal separation procedure

The maternal separation procedure has been recently described by Chocyk et al. (2011, 2013–2015). Briefly, on each of the PNDs 1–14, the dams and pups were removed from the maternity cages for 3 h (09.00–12.00). The mothers were placed individually in holding cages (Type III, 38 × 24 × 19 cm), while each litter was placed in a plastic container (22 × 16 × 10 cm) lined with fresh bedding material, moved to an adjacent room and placed in an incubator set at a constant temperature of 34 °C. After the 3 h separation, the pups and dams were returned to the maternity cages. AFR animals were left undisturbed with their mothers except during the weekly cage cleaning, indicating a small amount of handling. The impact of MS procedure on specific maternal and pup behaviors had been previously described in detail by our group (Chocyk et al., 2013). The animals were weaned on PND 22 and housed under controlled conditions (as described above) in standard plastic cages (Type IV, 57 × 33 × 20 cm) in groups of five of the same sex and under the same treatment protocol until adolescence (PND 35- PND 42). For the purposes of the present study only the adolescent females were subjected to further experimental procedures; the male subjects were used in other experiments.

2.1.2. Experimental group assessment

Female offspring coming from 16 AFR and 16 MS litters were used in the study. To avoid litter effects, the final experimental groups consisted of subjects that usually originated from different litters and were unrelated; in some rare cases (in behavioral experiments) two subjects from the same litter were used in specific experimental group (n = 6–12, i.e., subjects came from 6 to 8 different litters, see figure legends for details). Due to the potential impact of drug-induced behaviors on biochemical analyses, as well as possible interactions between different drugs and experimental procedures, separate groups of animals were used for: (a) immunohistochemical studies, (b) RT qPCR, (c) grooming behavior, (d) each experiment that studied dopamine receptor agonist-induced locomotion. Measurements of locomotor response to novelty (experimental procedure 1, see: Section 2.5) always preceded drug-induced behaviors (experimental procedure 2); therefore the data were culled from different experiments and, in consequence, the number of subject per group (n) reached 26–30 animals.

2.1.3. Drug treatments

Acute injections of R(−)-apomorphine hydrochloride (1 mg/kg s.c., Tocris) or its vehicle (0.1% ascorbic acid, 2 ml/kg, s.c.) were administered to study the effects of nonselective dopamine receptor agonist on locomotor activity.

To study the impact of selective D2-like dopamine receptor agonist on the locomotor activity, the rats received an acute injection of (−)-quinpirole hydrochloride (0.2 mg/kg s.c., Tocris) or its vehicle (saline, 1 ml/kg, s.c.).

To estimate the effects of selective D1-like dopamine receptor agonist on locomotion and grooming, the rats were injected with a