Neuroscience 277 (2014) 343–355

LONG-TERM EFFECTS OF CHRONIC COCAINE EXPOSURE THROUGHOUT ADOLESCENCE ON ANXIETY AND STRESS RESPONSIVITY IN A WISTAR RAT MODEL

C. J. ALVES,[a,b] A. MAGALHÃES,[a,d] P. MELO,[a,c] L. DE SOUSA,[d] M. A. TAVARES,[e] P. R. R. MONTEIRO[c†] AND T. SUMMAVIELLE[a,c†*]

[a] *IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Rua do Campo Alegre, 823, 4150-180 Porto, Portugal*

[b] *INEB – Instituto de Engenharia Biomédica, Universidade do Porto, Rua do Campo Alegre, 823, 4150-180 Porto, Portugal*

[c] *ESTSP – Escola Superior de Tecnologia da Saúde do Porto, Instituto Politécnico do Porto, Rua Valente Perfeito, 322, 4400-330 Vila Nova de Gaia, Portugal*

[d] *ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade de Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal*

[e] *FMUP – Faculdade de Medicina, Universidade do Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal*

Abstract—Adolescents display increased vulnerability to engage in drug experimentation. This is often considered a risk factor for later drug abuse. In this scenario, the permanent effects of cocaine exposure during adolescence on anxiety levels and stress responsivity, which may result in behavioral phenotypes prone to addiction, are now starting to be unveiled. Thus, the purpose of the present study was to evaluate the long-lasting effects of chronic cocaine administration during adolescence, on anxiety-like behavior and on stress response. Adolescent male Wistar rats were daily administered 45-mg cocaine/kg of body weight in three equal intraperitoneal doses with 1-h interval, from postnatal day (PND) 35 to 50. The effects of cocaine administration on anxiety levels, assessed in the Elevated Plus Maze (EPM), and on social stress response, assessed in the resident–intruder paradigm (R/I), were evaluated 10 days after withdrawal, when rats were reaching the adulthood. The underlying dopaminergic activity, and the corticosterone and testosterone levels were determined. Our results showed that cocaine induced long-lasting alterations in the hypothalamus–pituitary–adrenals (HPA) axis function and in testosterone levels. Such alterations resulted in significant and enduring changes in behavioral responses to environmental challenges, such as the EPM and R/I, including the evaluation of potential threats that may lead to high-risk behavior and low-benefit choices. This was further supported by an altered dopaminergic function in the amygdala and hippocampus. The present findings provide new insights into how the use of cocaine during adolescent development may modulate emotional behavior later in life. Compromised ability to recognize and deal with potential threats is an important risk factor to perpetuate compulsive drug seeking and relapse susceptibility.

Key words: adolescence, anxiety, cocaine, corticosterone, dopaminergic system, social stress.

INTRODUCTION

The complex and everlasting features of addiction determine its chronic nature, where the risk of relapse is present even after long withdrawal periods. In drug addiction, several risk factors were identified as promoters of relapse during withdrawal periods, and among these, high anxiety levels and stress are commonly reported (Shaham et al., 2000; Brown et al., 2012; Buffalari et al., 2012). As relapse is prevalent in recovery periods, a better understanding of the involved risk factors will promote the development of more effective treatment strategies in drug addiction.

The psychostimulant effects of cocaine are mainly due to its action on the mesocorticolimbic monoaminergic system (Ungless et al., 2001; Nogueira et al., 2006; Gu et al., 2010; Zhang et al., 2013), a neural pathway involved in the processing of affective information and emotional regulation (Kelley and Berridge, 2002). Exposure to cocaine induces a neuroadaptational process that will lead to altered emotional behavior (Young et al., 2011), such as increased anxiety levels and impaired stress responsivity. In cocaine abusers, high levels of anxiety were reported after cessation of cocaine use (Gawin and Kleber, 1986; Satel et al., 1991; Coffey et al., 2000). In animal studies, elevated anxiety levels were shown in withdrawal periods, either in initial stages (Harris and Aston-Jones, 1993; Sarnyai et al., 1995) or

*Correspondence to: T. Summavielle, Addiction Biology Group, Instituto de Biologia Molecular e Celular (IBMC), Rua do Campo Alegre, 823, 4150-180 Porto, Portugal. Tel: +351-226074900; fax: +351-226099157.

E-mail address: tsummavi@ibmc.up.pt (T. Summavielle).

† These authors contributed equally to the current manuscript.

Abbreviations: ANOVA, analysis of variance; DA, dopamine; DAT, dopamine transporter; DOPAC, 3,4-dihydroxyphenylacetic acid; EDTA, ethylenediaminetetraacetic acid; EPM, Elevated Plus Maze; GAPDH, glyceraldahyde-3-phosphate-dehydrogenase; HPA, hypothalamus–pituitary–adrenals; HVA, homovanillic acid/4-hydroxy-3-methoxyphenylacetic acid; Nac, nucleus accumbens; PND, postnatal day; qRT-PCR, quantitative real-time polymerase chain reaction; R/I, Resident–intruder paradigm; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TH, tyrosine hydroxylase; VTA/SN, ventral mesencephalon (comprising the ventral tegmental area and substantia nigra).

http://dx.doi.org/10.1016/j.neuroscience.2014.07.008

after long periods (Sobrian et al., 2003; Salas-Ramirez et al., 2010). Importantly, treatments that target the anxiety symptoms effectively reduced the risk of cocaine reinstatement (Buffalari et al., 2012), which supports the relevance of anxiety in the etiology of cocaine addiction.

In humans, there is also evidence supporting a link between stress exposure and addiction (Zaslav, 1994; Jacobsen et al., 2001; Mahoney et al., 2013). In rats, prior exposure to stressful stimuli was shown to result in facilitated acquisition (Piazza et al., 1990; Haney et al., 1995; Goeders and Guerin, 1996), maintenance (Miczek and Mutschler, 1996), and reinstatement of cocaine-seeking (Erb et al., 1998; Brown et al., 2012). Cocaine exposure was also shown to modify the stress response (Chaplin et al., 2010; Sithisarn et al., 2011). In agreement, abstinent cocaine-dependent individuals showed enhanced responsivity to stress (Fox et al., 2008). In adult rodents, cocaine-exposure also led to increased stress reactivity (Aujla et al., 2008), as evidenced by enhanced corticosterone response to both the forced swim and restraint stress test during the withdrawal period (Mantsch et al., 2007; Cleck et al., 2008).

Early drug use in the adolescence or young adulthood has been reported as a predictor of later drug abuse (Anthony and Petronis, 1995; Chen et al., 2009). In the adolescent, the undergoing psychophysiological changes (Spear, 2000; Giedd et al., 2009), may lead to increased susceptibility to the consequences of drugs of abuse (Spear, 2000; Andersen, 2003). The adolescent neurodevelopment is characterized by a period of profound structural maturation of the forebrain, associated to increased influence of the motivational drives for novel experiences, in a background of immature inhibitory control due to hypofunctionality of the medial prefrontal cortex (Chambers et al., 2003; Casey and Jones, 2010). This may predispose the adolescent to greater impulsivity and risk-taking, and increase the probability of drug experimentation (Spear, 2000; Chambers et al., 2003; Kuhn et al., 2013). Earlier behavioral studies using locomotor sensitization, conditioned place preference and self-administration paradigms, indicated that adolescents are more vulnerable to the detrimental effects, as well as to the rewarding and reinforcing properties, of addictive drugs (see reviews by Tirelli et al., 2003; Leslie et al., 2004; Barron et al., 2005). Increased vulnerability to cocaine reward during adolescence was recently further supported in an electrophysiological study by Wong et al. (2013), where reward unbalance in this stage is associated with heightened activity of the dopaminergic neurons.

A current major issue yet not fully addressed, is whether the action of addictive drugs in the adolescent developing brain will lead to enduring changes that may compromise adult behavior, increasing vulnerability to addiction (Kuhn et al., 2013). Recent imagiology studies in mice have shown that cocaine induces morphological changes in brain regions implicated in addiction that are more pronounced when exposure occurs in adolescence (Wheeler et al., 2013). The same study has also shown that cocaine-exposure in the adolescent led to increased locomotor sensitization in the adulthood (Wheeler et al., 2013), suggesting that cocaine-induced neuromorphological changes lead to persistent drug-related behaviors. Another recent study, reported a functional impairment of the prefrontal GABAergic network as a consequence of cocaine-exposure in the adolescence, resulting in a lasting disinhibition of the medial prefrontal cortex (Cass et al., 2013). Compromised medial prefrontal cortex function predicts the development of impulsive behavior and impaired decision-making, which are behavioral traits associated with increased susceptibility for substance abuse (Cass et al., 2013; Kuhn et al., 2013).

The present study aims to provide new insights into how the use of cocaine during adolescence may persistently affect stress response and anxiety and modulate behavioral responses later on. Altered response to environmental challenges representing potential threats, may lead to impaired risk-taking behavior and perpetuate compulsive drug seeking and relapse susceptibility. Therefore, in the present work, we used a rat model of chronic cocaine administration throughout adolescence to evaluate anxiety-like behavior and social stress responsivity in the Elevated Plus Maze (EPM) or the resident–intruder paradigm (R/I). The underlying corticosterone response and the testosterone levels were evaluated. The dopaminergic function in relevant brain regions was also assessed. All evaluations were conducted 10 days after the end of the administration period, when rats were reaching the adulthood.

EXPERIMENTAL PROCEDURES

Animal model

A total of 68 males born from primiparous three-month-old Wistar female rats acquired from Charles River Laboratories España S.A. (Barcelona, Spain) were used in this study. Animals were kept under stable conditions (20–22 °C, 60% humidity and 12-h light/dark cycle), with water and appropriate food supplied *ad libitum*. Cylindrical plastic tubes and soft paper for nest construction were made available to reduce stress. All procedures used were approved by local ethics committee and by the Portuguese Agency for Animal Welfare, general board of Veterinary Medicine, in compliance with the European Community Council Directive of September 22, 2010 (2010/63/UE). All procedures involving animals were conducted by FELASA C graded researchers, and all efforts were made to minimize the number of animals used and their suffering. Rats were randomly assigned to different experimental groups and treated following a binge pattern administration of cocaine from postnatal day (PND) 35 to PND 50. This administration period was selected to match the onset of mid-adolescence and extend into the late-adolescence period, during which intense reorganization of the mesocorticolimbic dopamine system is occurring (Andersen, 2003; Varlinskaya and Spear, 2008). These animals received three daily administrations of 15-mg cocaine/kg of body weight, administrated in a volume of 1 mL/kg and injected intraperitoneally every hour between 9:00 and 11:00 a.m. This dosing schedule was selected to mimic a frequent