Neuroscience 285 (2015) 34–46

SELF ADMINISTRATION OF OXYCODONE ALTERS SYNAPTIC PLASTICITY GENE EXPRESSION IN THE HIPPOCAMPUS DIFFERENTIALLY IN MALE ADOLESCENT AND ADULT MICE

Y. ZHANG,[a]* A. J. BROWNSTEIN,[a] M. BUONORA,[a] K. NIIKURA,[a] A. HO,[a] J. CORREA DA ROSA,[b] M. J. KREEK[a] AND J. OTT[c,d]

[a] *The Laboratory of the Biology of Addictive Diseases, The Rockefeller University, New York, NY 10065, USA*

[b] *Laboratory of Investigative Dermatology, The Rockefeller University, New York, NY 10065, USA*

[c] *Institute of Psychology, Chinese Academy of Sciences, Beijing, China*

[d] *The Laboratory of Statistical Genetics, The Rockefeller University, New York, NY, USA*

Abstract—Abuse and addiction to prescription opioids such as oxycodone (a short-acting Mu opioid receptor (MOP-r) agonist) in adolescence is a pressing public health issue. We have previously shown differences in oxycodone self-administration behaviors between adolescent and adult C57BL/6J mice and expression of striatal neurotransmitter receptor genes, in areas involved in reward. In this study, we aimed to determine whether oxycodone self-administration differentially affects genes regulating synaptic plasticity in the hippocampus of adolescent compared to adult mice, since the hippocampus may be involved in learning aspects associated with chronic drug self administration. Hippocampus was isolated for mRNA analysis from mice that had self administered oxycodone (0.25 mg/kg/infusion) 2 h/day for 14 consecutive days or from yoked saline controls. Gene expression was analyzed with real-time polymerase chain reaction (PCR) using a commercially available ''synaptic plasticity'' PCR array containing 84 genes. We found that adolescent and adult control mice significantly differed in the expression of several genes in the absence of oxycodone exposure, including those coding for mitogen-activated protein kinase, calcium/calmodulin-dependent protein kinase II gamma subunit, glutamate receptor, ionotropic AMPA2 and metabotropic 5. Chronic oxycodone self administration increased proviral integration site 1 (*Pim1*) and thymoma viral proto-oncogene 1 mRNA levels compared to controls in both age groups. Both *Pim1* and cadherin 2 mRNAs showed a significant combined effect of Drug Condition and Age × Drug Condition. Furthermore, the mRNA levels of both cadherin 2 and cAMP response element modulators showed an experiment-wise significant difference between oxycodone and saline control in adult but not in adolescent mice. Overall, this study demonstrates for the first time that chronic oxycodone self-administration differentially alters synaptic plasticity gene expression in the hippocampus of adolescent and adult mice.

Key words: oxycodone self-administration, hippocampus, synaptic plasticity, gene expression, adult, adolescent.

INTRODUCTION

Prescription opioid abuse poses a significant public health issue in the United States (Zosel et al., 2013). This problem has escalated over the past two decades, especially among adolescents (Compton and Volkow, 2006; Johnston et al., 2006; Johnston, 2009), of whom thousands are hospitalized every year as a result of non-medical use of prescription opioids (Zosel et al., 2013). This issue is particularly troublesome because little is known about how the rapidly changing adolescent brain is affected by exposure to prescription opioids (Compton and Volkow, 2006). As a result of the high degree of neuroplasticity during adolescence (Carpenter-Hyland and Chandler, 2007), the neurobiological alterations that adolescents experience in response to prescription opioids may be mechanistically different, or may persist into adulthood, thus conferring on them a greater vulnerability to opioid addiction upon subsequent abuse.

As drugs of abuse have been shown to engage the molecular mechanisms of learning and memory by affecting synaptic plasticity (Berke and Hyman, 2000; Kauer and Malenka, 2007), understanding the effects of addictive drugs on brain regions involved in learning and memory, such as the hippocampus (Billa et al., 2010) is crucial. The hippocampus has been shown to be integral to the storage, consolidation, and retrieval of declarative, spatial, and associative long-term memory (Hernandez-Rabaza et al., 2007), with long-term potentiation (LTP) and long-term depression (LTD) as representative measures of these processes (Caruana et al., 2012). Due to its role in learning and memory and through its efferent and afferent neural connections with the reward system (Kenney and Gould, 2008; Garcia-Fuster et al., 2011), the hippocampus is important to the response to addictive drugs. In particular, the hippocampus has been shown to

*Corresponding author. Address: The Laboratory of the Biology of Addictive Diseases, The Rockefeller University, 1230 York Avenue, Box 171, New York, NY 10065, USA. Tel: +1-212-327-8490; fax: +1-212-327-8574.
E-mail address: zhangyo@rockefeller.edu (Y. Zhang).
Abbreviations: LTP, long-term potentiation; MOP-r, Mu opioid receptor; PCR, polymerase chain reaction.

http://dx.doi.org/10.1016/j.neuroscience.2014.11.013
0306-4522/

be involved in context-drug associations (Kenney and Gould, 2008), reward-related response (Bao et al., 2007; Hernandez-Rabaza et al., 2007), as well as drug craving (Volkow, 2004) and seeking behavior (Vorel et al., 2001; Belujon and Grace, 2011), especially in the context of reinstatement and relapse to drugs of abuse (Robbins and Everitt, 2002; Hernandez-Rabaza et al., 2007; Belujon and Grace, 2011). Furthermore, due to its role in modulating the hypothalamic–pituitary-adrenal (HPA) axis, the hippocampus plays a key role in stress-induced drug-seeking behavior (Garcia-Fuster et al., 2011).

There have been studies showing that opiates, morphine in particular, alter gene expression in several brain regions in adult rodents (Wang et al., 1999; Nestler, 2001; Rodriguez Parkitna et al., 2004; Korostynski et al., 2006; Hassan et al., 2010). One such study, which involved morphine-induced conditioned place preference , showed changes in the expression of genes in the hippocampus involved in vesicular transport, neurotransmitter release, and receptor trafficking (Marie-Claire et al., 2007). However, there are few studies detailing the effects of prescription opioids on changes in gene expression in the adolescent brain (Ellgren et al., 2007). Furthermore, much of the research on the effects of drugs of abuse on the adolescent hippocampus has focused on nicotine, alcohol, and cannabis. For example, one study demonstrated that adolescent rats given alcohol showed more hippocampal changes in protein expression relative to adolescent controls compared to adult rats given alcohol (Hargreaves et al., 2009).

We recently reported that adolescent mice self-administered significantly less oxycodone than adult mice (in the same mice described in the study presented here) (Mayer-Blackwell et al., 2014). We hypothesized that there were differential neurobiological alterations in brain regions associated with reward between adolescent and adult mice as a consequence of oxycodone self-administration. In this study, we examined this hypothesis by measuring the expression of genes involved in synaptic plasticity in the hippocampus. This study is in agreement with our previous finding that adolescent mice that self-administered oxycodone experienced different changes in gene expression of neurotransmitter receptors in the dorsal striatum relative to controls than did adult mice that self-administered oxycodone (Mayer-Blackwell et al., 2014).

EXPERIMENTAL PROCEDURES

Subjects

Male adolescent and adult (4 or 11 weeks old on arrival, respectively) C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME, USA) were housed in groups up to five with free access to food and water in a light-(12:12-h light/dark cycle, light on at 7:00 pm and off at 7:00 am) and temperature-(25 °C) controlled room. Animal care and experimental procedures were conducted according to the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources Commission on Life Sciences 1996). The experimental protocols used were approved by the Institutional Animal Care and Use Committee of The Rockefeller University.

Self-administration procedure

Catheter implantation. Following acclimation for 7 days, the mice were anesthetized with a combination of xylazine (8.0 mg/kg i.p.) and ketamine (80 mg/kg i.p.). After shaving and application of a 70% alcohol and iodine preparatory solution, incisions were made in the midscapular region and anteromedial to the foreleg. A catheter of approximately 6 cm in length (ID: 0.31 mm, OD: 0.64 mm) (Helix Medical, Inc., Carpinteria, CA, USA) was passed subcutaneously from the dorsal to the ventral incision. A 22-gauge needle was inserted into the jugular vein to guide the catheter into the vein. The catheter was tied to the vein with surgical silk. Physiological saline was then flushed through the catheter to avoid clotting and the catheter then capped with a stopper. Antibiotic ointment was applied to the catheter exit wounds on the animal's back and forearm. Mice were individually housed after the surgery and were allowed 4 days of recovery (due to the limited period of adolescence in the mouse (Adriani and Laviola, 2004; Spear, 2000) before being placed in operant test chambers for the self-administration procedure.

Operant conditioning chambers. The self-administration chamber, ENV-307W (21.6 cm × 17.8 cm × 12.7 cm, Med. Associates, St. Albans, VT, USA), was located inside a larger sound attenuation chamber (Med. Associates). Each chamber contained a wall with two small holes (0.9-cm diameter, 4.2 cm apart, 1.5 cm from the floor of the chamber). One hole was defined as active, the other was inactive. When the photocell in the active hole was triggered by a nose-poke, an infusion pump (Med. Associates) delivered an oxycodone infusion of 20 μl/3 s from a 5-ml syringe. The syringe was connected by a swivel via Tygon tubing. The infusion pump and syringe were outside the chamber. During infusion, a cue light above the active hole was illuminated. Each injection was followed by a 20-s "time-out" period during which poking responses were recorded but had no programed consequences. All responses at the inactive hole were also recorded. Mice were tested during the dark phase of the diurnal cycle (all experiments were performed between 8:00 am and 12:00 pm).

Oxycodone self-administration. A 2-h self-administration session was conducted daily. Mice were weighed and heparinized saline solution (0.02 ml of 30 IU/ml) was used daily to flush the catheter to maintain patency. During self-administration sessions, mice in the oxycodone (Sigma, St. Louis, MO, USA) groups were placed in the self-administration chamber and a nose-poke through the active hole led to an infusion of oxycodone (0.25 mg/kg/infusion) under an FR1 schedule for 14 days. Drug volume was controlled by a computer to follow daily changes in body weight of individual animals. Mice in the control groups received yoked saline infusions during all sessions (saline was