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Strain differences in the expression of endocannabinoid genes and in cannabinoid receptor binding in the brain of Lewis and Fischer 344 rats

Santiago M. Coria [a,1], David Roura-Martínez [a,1], Marcos Ucha [a], María Amparo Assis [a], Miguel Miguéns [b], Carmen García-Lecumberri [a], Alejandro Higuera-Matas [a,*], Emilio Ambrosio [a]

[a] Department of Psychobiology, School of Psychology, UNED, C/ Juan del Rosal 10, 28040 Madrid, Spain
[b] Department of Basic Psychology I, School of Psychology, UNED, C/ Juan del Rosal 10, 28040 Madrid, Spain

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ABSTRACT

The Lewis (LEW) and Fischer 344 (F344) rat strains have been proposed as a model to study certain genetic influences on drug use. These strains differ in terms of the self-administration of several drugs, and in their expression of various components of the dopaminergic, glutamatergic, GABAergic and endogenous opioid neurotransmitter systems. As the endocannabinoid system is linked to these systems, we investigated whether these two strains exhibit differences in cannabinoid receptor binding and in the expression of cannabinoid-related genes. Quantitative autoradiography of [^3H]-CP 55,940 binding levels and real-time PCR assays were used. F344 rats displayed higher levels of cannabinoid receptor binding in the lateral globus pallidus and weaker *CNR1* gene expression in the prefrontal cortex (PFc) than LEW rats. Moreover, the *N*-acyl phosphatidylethanolamine-specific phospholipase D/fatty acid amide hydrolase ratio was greater in the PFc and NAcc of F344 rats. Our results suggest that the endocannabinoid system may be a mediator of the individual differences that exist in the susceptibility to the rewarding effects of drugs of abuse.

1. Introduction

Vulnerability to addiction is a complex trait that is largely influenced by genetic factors and indeed, twin studies suggest that about 50% of this vulnerability is heritable (Uhl et al., 2008). Several animal models have been used to investigate the genetic components of vulnerability to addiction, including the Lewis (LEW) and Fischer 344 (F344) inbred rat strains. LEW rats self-administer larger amounts of most drugs of abuse than F344 rats (see Kosten and Ambrosio (2002) for a review). In recent years, several neurochemical differences between these strains have been described that may explain their differential sensitivity to the rewarding effects of drugs of abuse (Herradon et al., 2003; Higuera-Matas et al., 2011; Martin et al., 2003; Rivera et al., 2013; Sanchez-Cardoso et al., 2007, 2009). In particular, there are several differences in parameters of the endogenous opioid system, demonstrating that binding to μ-opioid receptors is weaker in LEW rats than in F344 rats. These lower binding levels in LEW rats appeared in several areas such as the caudate-putamen (CPu), the nucleus accumbens (NAcc) or the ventral tegmental area (VTA), and could be related to the differential sensitivity to the rewarding effects of drugs of abuse displayed by these strains (Altarifi et al., 2012; Berrendero et al., 2010; Drakenberg et al., 2006; Le Merrer et al., 2009). By contrast, the functional activity of these receptors was comparable in both strains, with the exception of the cingulate cortex and NAcc core, where enhanced μ-opioid receptor activity was observed in LEW rats. μ opioid receptors in the anterior cingulate cortex have been related to placebo analgesia in rats (Zhang et al., 2013) so it could be suggested that the differential functional activity of these receptors between LEW and F344 rats may indicate potential differences in such effect. Indeed, other differences between LEW and F344 rats in pain-related behaviors have already been reported (Vit et al., 2006). In addition, the basal proenkephalin mRNA content is lower in LEW *versus* F344 rats (Sanchez-Cardoso et al., 2007). This different expression could contribute to a lower activity of the reward system (Duvauchelle et al., 1996) that would be compensated by the enhanced drug intake typically observed in this strain.

There is a close relationship between the opioid and endocannabinoid systems (Corchero et al., 2004; Fattore et al., 2004; Fattore et al., 2005; Manzanares et al., 1998; Parolaro et al., 2010; Scavone et al., 2013;

Abbreviations: DAGLα, diacylglycerol lipase alpha; F344, Fischer 344; FAAH, fatty acid amino hydrolase; LEW, Lewis; LGP, lateral globus pallidus; MAGL, monoacylglycerol lipase; NAcc, nucleus accumbens; NAPE-PLD, *N*-acyl phosphatidylethanolamine phospholipase D; PFc, prefrontal cortex; THC, Δ^9-tetrahydrocannabinol.

* Corresponding author at: Departamento de Psicobiología, Facultad de Psicología, UNED, C/Juan del Rosal 10, Madrid, Spain. Tel.: +34 913989689; fax: +34 913987491.
E-mail addresses: smcoria@bec.uned.es (S.M. Coria), droura1@alumno.uned.es (D. Roura-Martínez), marcosucha@bec.uned.es (M. Ucha), amparoassis@psi.uned.es (M.A. Assis), mmiguens@psi.uned.es (M. Miguéns), cglecumberri@psi.uned.es (C. García-Lecumberri), ahiguera@psi.uned.es (A. Higuera-Matas), eambrosio@psi.uned.es (E. Ambrosio).

[1] These authors contributed equally to this work.

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Vigano et al., 2005a,b). For example, CB_1 and μ-opioid receptors co-localize in multiple brain regions, such as the spinal cord (Salio et al., 2001), the nucleus accumbens (NAcc: Pickel et al., 2004) and in patches of the dorsal striatum (Rodriguez et al., 2001). In addition, there is compelling evidence that CB_1 and μ opioid receptors dimerize, both *in vitro* and *in vivo* (Hojo et al., 2008; Mackie, 2005; Rios et al., 2006; Schoffelmeer et al., 2006). Chronic administration of different cannabinoids was also associated to increased levels of proenkephalin gene expression (Manzanares et al., 1998). There are also reported interactions between both systems at the functional level, for example an intra-accumbal WIN 55,512-2 (WIN — a cannabinoid agonist) injection potentiated the conditioned place preference induced by morphine (Karimi et al., 2013). Cannabinoid receptor agonists also enhanced the antinociceptive effects of μ-opioid receptor agonists in rhesus monkeys without affecting opioid self-administration (Maguire et al., 2013). There are several more examples of such interactions that have been extensively reviewed elsewhere (Corchero et al., 2004; Fattore et al., 2005; Lopez-Moreno et al., 2010; Robledo et al., 2008; Scavone et al., 2013; Vigano et al., 2005a). Given the close relationship between these two systems, we recently carried out a series of experiments in which we analyzed the levels of endocannabinoid-related proteins in the hippocampus of LEW and F344 rats, both in naïve and saline-treated animals, and in those trained to self-administer cocaine (Rivera et al., 2013). LEW rats exhibited weaker CB_1 expression but stronger CB_2 expression than F344 rats. Considering that CB_1 activation has well documented amnestic effects (Han et al., 2012; Puighermanal et al., 2009; Robinson et al., 2008), this differential CB_1 expression might explain, at least to some degree, the worse performance in a radial maze spatial learning and memory task displayed by F344 rats as compared to LEW rats (Fole et al., 2011; van der Staay et al., 2009). Hippocampal CB_2 receptors have been involved in aversive memory consolidation (Garcia-Gutierrez et al., 2013), so it would be interesting to determine if the higher levels of these proteins in F344 rats are associated to enhanced consolidation of such memories in this strain. Furthermore, the *N*-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD)/fatty acid amino hydrolase (FAAH) ratio was higher in the stratum pyramidale of the CA3 hippocampal field of F344 compared to that in LEW rats. This increased ratio might be indicative of higher anandamide levels. If this were the case it could contribute to explain the higher anxiety found in the F344 strain (Busquets-Garcia et al., 2011; Skripuletz et al., 2010).

To expand upon these latter findings, in the present study we used quantitative autoradiography to measure cannabinoid receptor binding throughout several regions of the encephalon of LEW and F344 rats. In a separate set of animals, we also measured the mRNA expression of several endocannabinoid-related proteins (receptors and enzymes) in the NAcc and PFc, regions that represent important nodes in the reward system. More specifically, we analyzed the expression of genes encoding the enzymes that mediate the synthesis and degradation of anandamide (NAPE-PLD and FAAH, respectively) and of 2-arachidonoyl glycerol (2-AG: diacylglycerol lipase alpha [DAGLα] and monoacylglycerol lipase [MAGL], respectively), as well as those encoding the CB_1 and GPR55 receptors. F344 rats displayed higher cannabinoid receptor binding in the lateral globus pallidus (LGP) than their LEW counterparts. In addition, there were strain-specific effects on the expression of the *CNR1* (coding for the CB_1 receptor) *FAAH* and *MGLL* (coding for the MAGL enzyme) genes in the PFc and, in the case of the *FAAH* in the NAcc as well. Conversely, there were no changes in DAGLα or GPR55 gene expression.

These data add to the growing body of literature showing the involvement of the endocannabinoid system in the individual differences in the sensitivity to the rewarding properties of drugs of abuse.

2. Experimental procedures

2.1. Animals

Male F344 and LEW rats weighing 250–275 g at the beginning of the experiments were used in these studies (initial sample size: LEW, n =

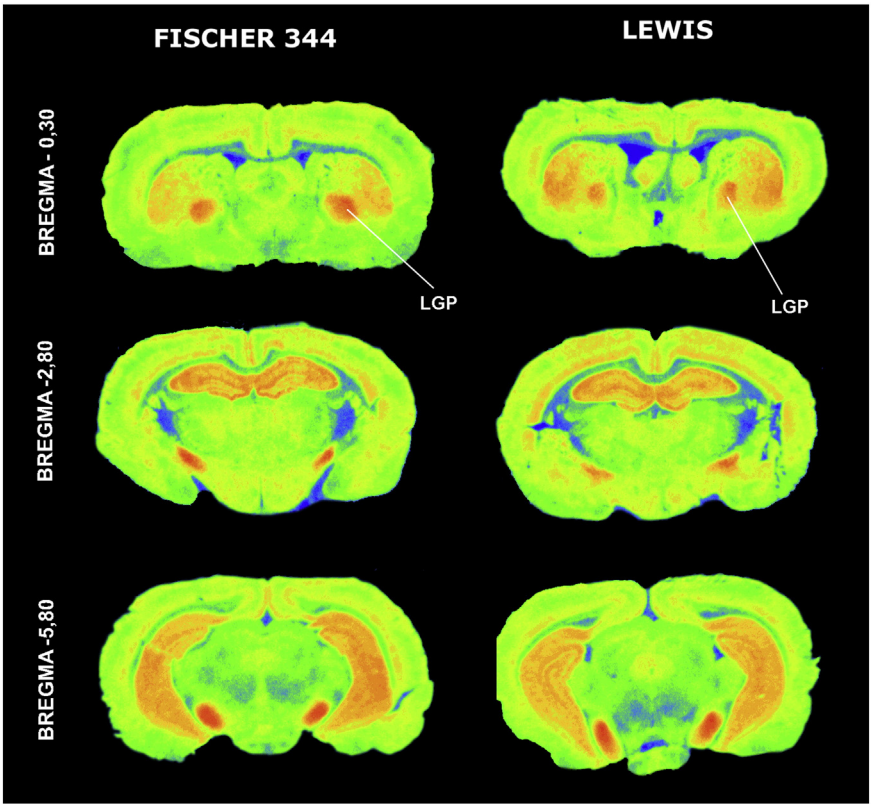

Fig. 1. Representative autoradiographs of total [^3H]-CP 55.940 binding to cannabinoid receptors at 3 different encephalic levels. Note the more intense binding in the LGP of F344 rats.