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# Sex-dependent effects of periadolescent exposure to the cannabinoid agonist CP-55,940 on morphine self-administration behaviour and the endogenous opioid system

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**Abstract**

Early cannabinoid consumption may predispose individuals to the misuse of addictive drugs later in life. However, there is a lack of experimental evidence as to whether cannabinoid exposure during adolescence might differently affect opiate reinforcing efficacy and the opioid system in adults of both sexes. Our aim was to examine whether periadolescent chronic exposure to the cannabinoid agonist CP-55,940 could exert sex-dependent effects on morphine reinforcing and the opioid system in adulthood. Morphine reinforcing was studied under a progressive ratio (PR) reinforcement schedule in adult male and female rats that previously acquired morphine self-administration under a fixed ratio 1 (FR1) schedule. Binding levels and functionality of μ-opioid receptors were also evaluated. Periadolescent cannabinoid exposure altered morphine self-administration and the opioid system in adult rats in a sex-dependent manner. CP-55,940-exposed males exhibited higher self-administration rates under a FR1, but not under a PR schedule. In females, CP-55,940 did not modify morphine self-administration under either schedule. Moreover, CP-55,940 also increased μ-opioid receptor levels in the subcallosal streak of pre-treated animals and decreased μ-opioid receptor functionality in the nucleus accumbens shell but again, only in males. Our data indicate that adult male rats exposed to the cannabinoid in adolescence self-administer more morphine than females, but only when the demands required by the schedule of reinforcement are low, which might be related to the decrease in μ-opioid receptor functionality in the NAcc-shell observed in these animals.


*Keywords:* Adolescence; Cannabis; Opiate; Self-administration; μ-Opioid receptors; Autoradiography

## 1. Introduction

Several studies have consistently shown that cannabis use has extended greatly in recent years in many developed societies, especially among adolescents (Gruber and Pope, 2002; Hall, 2006). It has also been reported that adolescents initiate cannabis use before consuming other illicit drugs. Moreover, there is a higher risk of starting using illicit drugs in younger marijuana initiates than in older ones (Yamaguchi and Kandel, 1984). Indeed, as the frequency of cannabis use increases, so does the risk of initiation into the consumption of other drugs of abuse (Kandel et al., 1992; Fergusson and Horwood, 2000). In an attempt to explain the strong association between cannabis use and that of other drugs of abuse different hypotheses have been proposed. It has been argued that a common factor, generically referred to as an individual's propensity for drug use, could influence the consumption of both cannabinoids and other illicit drugs (MacCoun, 1998; Morral et al., 2002). However, the idea that a causal link could exist has also been raised and thus, cannabinoids might promote the misuse of addictive drugs later in life (''gateway theory'') (Kandel et al., 1992; Fergusson and Horwood, 2000; Lynskey et al.,

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2003). Therefore, further experimental evidence is needed to support such proposals.

Results from animal studies have provided data about the existence of interactions between the effects of cannabinoids and other drugs of abuse in different experimental models (Manzanares et al., 1999; Fattore et al., 2005; Maldonado et al., 2006; Viveros et al., 2006 for reviews). However, only a few studies have tested the long-term effects of cannabinoids following exposure in the early stages of development and adolescence. In most studies cannabinoids have been administered in prenatal or perinatal stages. Accordingly, perinatal exposure to delta-9-tetrahydrocannabinol (THC) facilitates morphine-induced place preference conditioning (Rubio et al., 1998), increases morphine self-administration and changes μ-opioid receptor binding in several brain regions in adulthood (Vela et al., 1998). Perinatal THC also provokes a decrease in PENK gene expression in the caudate putamen of adult rats (Corchero et al., 1998). More recently, Spano et al. (2007) have shown that prenatal THC modifies several parameters of heroin self-administration and increases PENK mRNA expression in adulthood. Moreover, in what we believe to be the first study of the effects of cannabinoid treatment in adolescence on drug self-administration in adulthood (Ellgren et al., 2007), exposure to THC in adolescent animals produced an increase in heroin self-administration, preproenkephalin mRNA expression and the functionality of μ-opioid receptors in adulthood.

However, experimental evidence is still lacking to assess whether pre-exposure to cannabinoids in adolescence might alter the reinforcing efficacy of drugs of abuse by using appropriate operant reinforcement schedules, such as PR schedules (Hodos, 1961; Arnold and Roberts, 1997). Indeed, differential effects of cannabinoids on drug self-administration seem to exist depending on the reinforcement schedule used (Vela et al., 1998; Ambrosio et al., 1999; González et al., 2003, 2004; Solinas et al., 2003, 2004 Panlilio et al., 2007). Thus, increased opiate self-administration was found in animals pre-exposed to THC under a FR1 schedule (Vela et al., 1998; Solinas et al., 2004), but not under a PR schedule (Ambrosio et al., 1999; González et al., 2003, 2004; Solinas et al., 2004). Since the response requirements are higher in the PR than in the FR1 schedules, animals have to work harder to obtain the drug. Thus, these schedules provide more information about the magnitude of reinforcing efficacy (i.e. motivational properties) of a particular drug when compared to the FR1 schedule, which is useful to determine whether a drug is reinforcing or not (Hodos, 1961; Arnold and Roberts, 1997). Consequently, PR schedules could better reveal whether the motivational properties of addictive drugs are really altered by cannabinoids. In order to further clarify the role that adolescent experience with cannabinoids could play in promoting subsequent drug misuse in adulthood, we have studied whether chronic periadolescent exposure to the cannabinoid agonist CP-55,940 could affect morphine reinforcing under a PR schedule of reinforcement in adult animals that previously acquired morphine self-administration behaviour under a FR1 schedule. We also evaluated whether periadolescent cannabinoid treatment induces perdurable changes in binding levels and functionality of μ-opioid receptors in adulthood.

In addition, there are no studies regarding the possible differential effects of cannabinoid exposure in adolescence on drug self-administration and neurotransmitter systems in adulthood depending on the sex. Although gender differences in the vulnerability to drug addiction are well documented (see Roth et al., 2004; Lynch, 2006 for reviews), most of the experimental research on cannabinoid–opiate interaction studies has been carried out on males (Norwood et al., 2003; Schneider and Koch, 2003; Solinas et al., 2004; Ellgren et al., 2007; Spano et al., 2007). We think that this is an important issue to address, because we and others have found sex differences in several neurochemical and behavioural responses to cannabinoid pre-exposure, including morphine self-administration (Vela et al., 1998; Corchero et al., 1998; Ambrosio et al., 1999; González et al., 2003; Biscaia et al., 2003). Therefore, we studied both male and female rats in the present work.

## 2. Materials and methods

### 2.1. Animals and treatments

Male and female Wistar albino rats (*Rattus norvegicus albinus*) were used in this study, the offspring of rats purchased from Harlan Interfauna Ibérica (Barcelona, Spain) that were mated (one male × one female) in our laboratory approximately 3 weeks after their arrival. All animals were maintained at a constant temperature (21 ± 1 °C) and in a reverse 12 h/12 h dark/light cycle (lights on at 20:00) with free access to food (Panlab, Barcelona, Spain) and water. On the day of birth (postnatal day 0), litters were sex-balanced and culled to obtain 10 ± 1 pups per dam. The animals were weaned at 22 days of age. All experimental procedures and animal care was in compliance with the European Union Guidelines on Laboratory Animal Care (Directive 86/609/EEC).

The synthetic cannabinoid receptor agonist CP-55,940 (0.4 mg/kg, 2 ml/kg i.p.; Tocris, Spain) or its corresponding vehicle (VEH, ethanol:cremophor:saline 1:1:18, cremophor; Fluka BioChemiKa) was administered once daily from the 35th to the 45th postnatal day to male and female rats. This exposure period was chosen since cannabinoid binding levels have been reported to reach maximum values at approximately this time (Rodríguez de Fonseca et al., 1993). At adulthood (10 weeks old), four groups of animals were then submitted to a self-administration study for about 2 weeks. Animals were randomly assigned to the following groups: CP-55,940-exposed males ($n = 9$), CP-55,940-exposed females ($n = 11$), VEH-exposed males ($n = 11$) and VEH-exposed females ($n = 10$). In addition, another four groups of rats were sacrificed at 12 weeks old without having undergone a self-administration procedure: CP-55,940-exposed males ($n = 6$), CP-55,940-exposed females ($n = 8$), VEH-exposed males ($n = 8$) and VEH-exposed females ($n = 8$). After sacrificing the animals, their brains were quickly removed and immersed in an isopentane bath (approximately −40 °C) for 1 min, then stored at −70 °C until quantitative and functional autoradiographic and in situ hybridization studies were carried out.

### 2.2. Operant morphine-self-administration study

#### 2.2.1. Surgery

An i.v. catheter of polyvinylchloride tubing (0.064″ i.d.) was surgically implanted into the right jugular vein of adult animals (10 weeks old), approximately at the level of the atrium, under ketamine (40 mg/kg i.p.) and diazepam (10 mg/kg i.p.) anaesthesia. The catheter passed s.c. and exited in the midscapular region before passing through a spring tether system (Alice King Chatham, USA) that was mounted to the skull of the rat with dental