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Social housing conditions influence morphine dependence and the extinction of morphine place preference in adolescent mice

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ABSTRACT

Background: Adolescent opioid abuse is on the rise, and current treatments are not effective in reducing rates of relapse. Our previous studies demonstrated that social housing conditions alter the acquisition rate of morphine conditioned place preference (CPP) in adolescent mice. Specifically, the acquisition rate of morphine CPP is slower in morphine-treated animals housed with drug-naïve animals. Thus, here we tested the effect of social housing conditions on the development of morphine dependence and the extinction rate of an acquired morphine CPP.
Methods: Adolescent male mice were group-housed in one of two housing conditions. They were injected for 6 days (PND 28-33) with 20 mg/kg morphine. Morphine only mice are animals where all four mice in the cage received morphine. Morphine cage-mate mice are morphine-injected animals housed with drug-naïve animals. Mice were individually tested for spontaneous withdrawal signs by quantifying jumping behavior 4, 8, 24, and 48 h after the final morphine injection. Then, mice were conditioned to acquire morphine CPP and were tested for the rate of extinction.
Results: Morphine cage-mates express less jumping behavior during morphine withdrawal as compared to morphine only mice. As expected, morphine cage-mate animals acquired morphine CPP more slowly than the morphine only animals. Additionally, morphine cage-mates extinguished morphine CPP more readily than morphine only mice.
Conclusions: Social housing conditions modulate morphine dependence and the extinction rate of morphine CPP. Extinction testing is relevant to human addiction because rehabilitations like extinction therapy may be used to aid human addicts in maintaining abstinence from drug use.

1. Introduction

Adolescence is a period of profound developmental changes. Among these changes are increases in risk-taking and novelty-seeking behaviors, which occur in both humans and non-human animals (Spear, 2000). Additionally, several physical and cognitive changes occur that may increase the adolescent's susceptibility to delinquent behaviors, including drug experimentation. For example, both the prefrontal cortex and the striatum, as well as the connectivity between them, were implicated in adolescents' increase in risky behaviors, including drug experimentation (Casey and Jones, 2010; Dewitt et al., 2014; Raznahan et al., 2014; Urosevic et al., 2014a, 2014b). Indeed, adolescence is when most people initiate drug use, as statistics show that many addicts report initial drug use between the ages of 12 and 14 (SAMSHA, 2011). Moreover, abuse of opioids, including prescription pain medications, has increased dramatically in this population (Johnston et al., 2013), with many more beginning to use every day. The percentage of adolescents seeking treatment for prescription opioids has increased from 15.5% to 34.5% in the past decade, and from 16.6% to 25.8% for heroin dependence (Johnston et al., 2013). In preclinical studies, social factors have been reported to modulate both the initiation and maintenance of drug abuse. However, much of this work has been done in adult animals, which neglects the at-risk demographic of adolescents.

One common problem that adolescent opioid addicts report is the feeling of being isolated and having a lack of social support (Hyucksun Shin, 2012; Vaughn et al., 2012). Reestablishing support from family and friends has often been described as a factor that aids in cessation of opioid use. Similar to humans, social interaction

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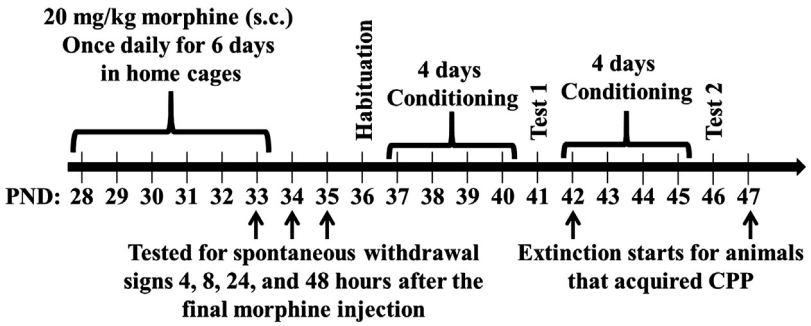

Fig. 1. A diagram summarizing the injection schedule of morphine in their home cages and the following experimental design.

is rewarding for adolescent rodents, and can be used to establish conditioned place preference (CPP; Douglas et al., 2004; Peartree et al., 2012). Moreover, the susceptibility to acquire drug-induced reward in adolescent rodents was demonstrated to be modulated by both social interactions in their home cages as well as social interaction during the acquisition of CPP. Synergistic interaction was observed between social reward and sub-threshold levels of cocaine and nicotine, i.e., levels of the drugs that by themselves could not induce conditioned place preference (Thiel et al., 2008, 2009). However, social enrichment in their home cages decreases cocaine CPP in adolescent rats, when levels of drug sufficient to establish CPP by itself were examined (Zakharova et al., 2009). These effects might be drug dependent given that social conditions did not alter the acquisition of amphetamine CPP in adolescent rats (Bowling and Bardo, 1994). Similarly, a complex interaction was observed between social enrichment in their home cages, social reward, and the establishment of morphine reward in adolescent mice (Kennedy et al., 2012). Additionally, the effects of social context on the acquisition of morphine reward were suggested to be influenced by genetic factors, given that different effects were observed in various mice strains.

Likewise, previous results from our lab have demonstrated that social housing conditions alter the rewarding properties of morphine during adolescence. We have previously shown that exposure to drug-naïve animals attenuates the acquisition of CPP to morphine (Cole et al., 2013). Specifically, morphine-treated rodents housed solely with drug-exposed cage-mates (i.e., morphine only mice) require fewer exposures to morphine (in the CPP apparatus) and a smaller dose of morphine to acquire morphine CPP as compared to morphine-treated rodents housed with drug-naïve cage-mates (i.e., morphine cage-mates). Thus, being housed with drug-naïve animals has a protective effect on the acquisition of morphine CPP.

To extend these observations, this study examined the effect of social housing on the extinction rate of morphine CPP. Extinction is a process in which a conditioned response gradually diminishes over time as an animal learns to uncouple a response from a stimulus (Peters et al., 2009; Quirk and Mueller, 2008). It is believed that it leads to the formation of a new, inhibitory memory that becomes expressed instead of the previous memory (Ma et al., 2012; Pavlov, 1927). In terms of CPP, the memory of the morphine reward is the stimulus that motivates animals to seek out the morphine-paired chamber (Bardo et al., 1984; Blander et al., 1984; Mucha and Iversen, 1984; Mucha et al., 1982; van der Kooy et al., 1982). Therefore, animals with memories that are less robust will most likely extinguish an acquired place preference more quickly than those with a strong memory. Thus, a further advantage of using the extinction paradigm is extending the quantitative assessment of the CPP model (Rutten et al., 2011). Additionally, the animals were examined for the effect of social housing on morphine dependence by monitoring spontaneous withdrawal symptoms. This is because the negative reinforcement that results from aversive symptoms can enhance incentive value of a drug and maintenance of drug seeking behavior (Koob, 1992; Schulteis and Koob, 1994).

2. Methods and materials

2.1. Animals

All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee. Adolescent male C57BL/6N mice were purchased from Harlan Lab (Houston, TX, USA) and housed four per cage with food and water *ad lib* in a temperature-controlled (21+/−2 °C, humidity 45%) vivarium with a 12-h light/12-h dark cycle (light on at 7:30 AM). All mice were housed as cage-mates from the day of weaning (postnatal day (PND) 21) in Harlan facilities, shipped as cage-mates and remained cage-mates after arriving at our facility and for the entire experiment. Mice were purchased at PND 22, acclimated to the vivarium until PND 28, and injected with morphine from PND 28 to 33, when the behavioral testing started. Thus, mice began opioid injections during what is considered the late phase of their prepubescent period, and were tested during their mid-adolescence/periadolescent period (reviewed in Spear, 2000).

2.2. Housing conditions

Mice were group-housed four per cage in one of two conditions. Morphine only mice are animals where all four mice in the cage received morphine and were housed physically and visually separated from drug-naïve animals. Morphine cage-mate mice are morphine-injected animals housed with drug-naïve animals (i.e., two mice in the cage received morphine, two mice received saline).

2.3. Morphine pretreatment regimen

Mice were injected once daily (9:00 AM) in their home cage for six consecutive days with 20 mg/kg of morphine sulfate (subcutaneously, Sigma-Aldrich, St. Louis, MO, USA), or saline (10 ml/kg) for a total of six injections. The injection schedule and the following experimental design are summarized in Fig. 1.

2.4. Spontaneous withdrawal symptoms

Morphine-injected animals were tested 4, 8, 24, and 48 h following the final morphine injection (Fig. 1). They were individually placed in Plexiglas cylinders (37 cm tall × 14.5 cm in diameter) and were videotaped for 30 min. The videotapes were scored for jumping behavior by an observer who was blind to treatment conditions.

2.5. CPP apparatus

Morphine CPP study was conducted in a set of eight identical apparatuses. Each Plexiglas CPP apparatus contained three 20 × 20 × 30.5 cm^3 chambers. Two of the chambers were used for discrimination, and contained distinct olfactory and visual cues, while the third chamber was neutral, and contained no olfactory or visual cues. One of the chambers was covered in a checkered pattern, and was scented with almond extract (Adams Extract and Spices, LLC, Gonzales, TX, USA) as an olfactory cue 5 min before the beginning of the session. The other chamber was covered in a cow pattern and was scented with lemon extract (Adams Extract and Spices, LLC, Gonzales, TX, USA), also placed in the chamber 5 min before the beginning of the session. The 200 μl scents were placed on a filter paper that was hung in the top corner of the chamber. The visual cues contained approximately equal amounts of white and black. On habituation and test sessions, two doors allowed free access between the three chambers, while on conditioning days, animals were confined to one of the chambers. Also, on habituation and test sessions, the animals were placed directly into the neutral chamber. Each day, mice were habituated to the