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# The antipsychotic aripiprazole selectively prevents the stimulant and rewarding effects of morphine in mice

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ABSTRACT

Aripiprazole is an antipsychotic that acts as a partial agonist at dopamine $D_2$ receptors, with a favorable pharmacological profile. Due to its unique mechanism of action, this compound has potential application as a substitutive therapy for drug addiction. Considering that distinct neural systems subserve the addictive and analgesic actions of opioids, we tested the hypothesis that aripiprazole selectively inhibit the abuse-related, but not the antinociceptive, effects of morphine. The drugs were tested in male Swiss mice for their effects on locomotion, conditioned place preference (CPP) and nociception. Morphine (20 mg/kg) increased motor activity, whereas aripiprazole (0.1, 1 and 10 mg/kg) did not induce any change. This antipsychotic, however, prevented morphine-induced locomotion. In the conditioning box, aripiprazole did not induce either reward or aversion. Yet, it prevented both the acquisition and the expression of morphine-induced CPP. Finally, none of the doses of this antipsychotic interfere with morphine (5 mg/kg)-induced antinociception in the tail-flick test. In conclusion, aripiprazole inhibited the abuse-related effects of morphine at doses that do not interfere with basal locomotion, reward or aversion. Also, it did not alter morphine-induced antinociceptive effects. This antipsychotic should be further investigated as a possible substitutive strategy for treating certain aspects of opioid addiction.



## 1. Introduction

Drug addiction is a chronic, multifactorial psychiatric disorder, potentially relapsing, in which there is a compulsion to self-administer substances at all costs (Cami and Farre, 2003; Everitt and Robbins, 2005; Justinova et al., 2009). Of particular importance are the opioid compounds, a group of analgesic and addictive drugs that include heroin and morphine (Compton and Volkow, 2006). Morphine has its medical use mainly in the treatment of moderate-to-severe pain (W.H.O., 1992). It acts as a full agonist at μ opioid receptors and as a partial agonist at κ receptors (Adunsky et al., 2002; Maddocks et al., 1996).

The remarkable addictive potential of morphine results, at least in part, from μ opioid receptor agonism, followed by activation of the dopamine-mediated neurotransmission in the mesolimbic pathway, which has been proposed as a convergent mechanism of most abused drugs (Bozarth and Wise, 1982; Di Chiara, 1999; Koob, 1992; Koob and Volkow, 2010; Matthews and German, 1984; Torigoe et al., 2012; Wise and Bozarth, 1982). Thus, substances that act as antagonists at dopamine $D_{2/3}$ receptors, namely the antipsychotic drugs, have been used to attenuate central reactions to morphine, including delusions and hallucinations (McNicol et al., 2003). They have also been considered as "antagonist therapies", since they prevent the rewarding and reinforcing effects of drugs of abuse (O'Brien, 2008; Pierce et al., 2012; Torigoe et al., 2012).

The efficacy of this strategy is limited, however, by the adverse effects of the antipsychotic drugs, including motor impairment, and by the low adherence, since this class of pharmaceuticals can induce aversive reactions (Modell et al., 1993; Swegle and Logemann, 2006). Thus, a more effective pharmacological strategy could be the use of antipsychotic drugs that act as partial agonists at the dopamine receptors. Aripiprazole is an antipsychotic that exerts its effects, at least in part, through this mechanism (Burris et al., 2002; Jordan et al., 2002). Its complex pharmacology is thought to entail partial agonism at dopamine $D_2$ and serotonin (5-hydroxytryptamine, 5-HT) 5-$HT_{1A}$ receptors in addition to antagonism at 5-$HT_{2A}$ receptors (DeLeon et al., 2004; Kane et al., 2002; Marder et al., 2003; Shapiro et al., 2003; Stark et al., 2007). This compound has been considered as a new tool for a pharmacological

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approach aimed to minimize the effects of drugs of abuse, since it might reduce the rewarding effects of drugs without inducing aversive states by itself (Ohlsen and Pilowsky, 2005).

In line with this notion, this antipsychotic decrease methamphetamine and cocaine self-administration (Thomsen et al., 2008; Wee et al., 2007) and prevent the motor hyperactivity ("hyperlocomotion") induced by psychostimulant drugs in experimental animals (Leite et al., 2008). It also selectively inhibited the stimulant effects of ethanol, without exacerbating the motor impairment induced by this drug (Viana et al., 2013). Considering this evidence, the present study was designed to test the hypothesis that aripiprazole selectively prevents morphine-induced hyperactivity and conditioned place preference (CPP), two behavioral tests widely used to study drug rewarding effects (Bardo and Bevins, 2000; Cunningham et al., 2006; Sanchis-Segura and Spanagel, 2006). We reasoned that this antipsychotic could exert such effects without interfering with the analgesic properties of morphine.

## 2. Materials and methods

### 2.1. Experimental animals

Male Swiss mice (20–25 g) were housed in a room maintained at $25 \pm 1$ °C with a 12 h light/dark cycle. Food and water were available ad libitum. Each animal was used only once. The present study was approved by the Committee for Ethics in Animal Experimentation (CETEA) under the protocol number 109/2011. All protocols were conducted in accordance with the Brazilian Society of Neuroscience and Behavior Guidelines for the Care and Use of Laboratory Animals. Every effort was made to minimize animal suffering.

### 2.2. Drugs

Aripiprazole (0.1, 1 and 10 mg/kg; powder, kindly provided by Brystol-Myers Squibb and Otsuka Pharmaceuticals) was dissolved in physiological saline containing tween-80 at 5% (Viana et al., 2013). Morphine hydrochloride (1.25, 2.5, 5, 10 and 20 mg/kg; powder, bought from Tocris) was diluted in physiological saline (Romero et al., 2011). The solutions were prepared immediately before use and injected via intraperitoneal route in a volume of 10 mL/kg.

### 2.3. Apparatus and procedures

All the experiments were conducted in the light phase, between 8.00 and 11.30 a.m. in an isolated, sound-attenuated room under illumination of 200 lx. The experiments measuring locomotion were carried out in a circular open field or arena (40 cm in diameter with a 50 cm high Plexiglas wall). The apparatus was video-recorded and the distance moved was automatically analyzed during 10 min with the help of the AnyMaze software.

CPP was assessed in a dark acrylic box consisting of two chambers, with equal sizes (20 cm long, 15 wide and 10 cm high) with doors ($5 \times 5$ cm) connecting them to a central compartment (6 cm long, 15 cm wide and 10 cm high). The walls of the lateral chambers were painted with interspersed black and white stripes and the floors consisted of removable metal surfaces. In one of the chambers (chamber A) the walls were painted with vertical stripes and the floor consisted of a metal grid with parallel, equally-spaced rods. The other (chamber B) had walls painted with horizontal stripes and a metal floor with circular holes. The lit intensity was similar among the three compartments. The apparatus was video recorded and the time spent by the animals in each compartment was automatically analyzed with the AnyMaze software.

The CPP protocol was randomized, unbiased and counterbalanced, consisting of three phases, namely pre-conditioning, conditioning and test. The animals were randomly assigned to one of the drug treatments. Then, in the pre-conditioning phase (first day), each mouse was placed in the central compartment of the box, with the doors open, and could freely explore the box during 30 min. The time spent in each compartment was registered. To avoid bias, it was established that the animals exhibiting preference for exploring one of the chambers (more than 70% of the time) would not be included in the experiments. In any case, there was no preference for none of the sides (i.e., this is an "unbiased" CPP protocol). In the conditioning phase (days 2–7), the animals were randomly assigned to one of the experimental treatments. They received drug injections on days 2, 4 and 6 and were immediately confined in one of the chambers (drug-paired side) for 30 min. Even though there was no preference for any of the chambers, half of the animals in each experimental group were confined in chamber A and the others in chamber B (i.e., this is a counterbalanced CPP protocol). On days 3, 5 and 7 vehicle was administered and the animals were confined in the alternate chamber for the same time. Finally, on the test phase (day 8), each animal was placed in the central compartment, with the doors open, and remained in the box for 30 min, when the time spent in each chamber was registered. The CPP index as calculated for each animal as the time spent in drug-paired side (chamber A or B, depending on the animal) during the test subtracted by the time spent in the same side in the pre-exposure phase (Cunningham et al., 2006).

The nociceptive responses were evaluated in the tail-flick test, which consisted in a heat source applied 2 cm from the tip of the tail. The protocol was conducted as previously described (D'amor and Smith, 1941). Briefly, following restraining of the mouse by one of experimenter's hand, a heat source was applied and the time(s) required for the animal to withdraw its tail from the heat source was recorded by an observed that was not aware of the treatments. The intensity of the heat was adjusted so that the baseline latencies were between 3 and 4 s. To avoid tissue damage and for ethical reasons, the cut-off time was established at 9 s. The baseline latency was obtained for each animal, before drug administration (zero time), by calculating an average of three consecutive trials. To reduce stress, mice were habituated to the apparatus 1 day prior to conducting the experiments (Romero et al., 2013).

### 2.4. Experiments

The aim of the first experiment was to test if aripiprazole (0.1, 1 and 10 mg/kg) alters basal locomotion. The animals received injection of vehicle or aripiprazole and were placed in the open field 15 min later. In the second experiment, to determine the effective dose of morphine, the animals received vehicle or drug (10 or 20 mg/kg) and were immediately placed in the open-field. In the third experiment, to test the hypothesis that aripiprazole prevents morphine-induced hyperlocomotion, the animals received an injection of vehicle or aripiprazole (0.1, 1 or 10 mg/kg) followed 15 min later by vehicle or morphine injection. Immediately after this, they were placed in the open field (Leite et al., 2008).

The fourth experiment was designed to evaluate if aripiprazole would induce rewarding or aversive effects. In the conditioning phase, the mice received injections of vehicle, morphine (20 mg/kg) or aripiprazole (0.1, 1 and 10 mg/kg), alternated with vehicle injections, accordingly to the protocol described above. No drug was injected in the test day. In the fifth experiment, we tested the