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Cocaine-conditioned place preference is predicted by previous anxiety-like behavior and is related to an increased number of neurons in the basolateral amygdala

David Ladrón de Guevara-Miranda[a], Francisco J. Pavón[b], Antonia Serrano[b], Patricia Rivera[b], Guillermo Estivill-Torrús[c], Juan Suárez[b], Fernando Rodríguez de Fonseca[b], Luis J. Santín[a,*], Estela Castilla-Ortega[b,**]

[a] Departamento de Psicobiología y Metodología de las Ciencias del Comportamiento, Instituto de Investigación Biomédica de Málaga (IBIMA), Facultad de Psicología, Universidad de Málaga, Spain
[b] Unidad de Gestión Clínica de Salud Mental, Instituto de Investigación Biomédica de Málaga (IBIMA), Hospital Regional Universitario de Málaga, Spain
[c] Unidad de Gestión Clínica Intercentros de Neurociencias, Instituto de Investigación Biomédica de Málaga (IBIMA), Hospitales Universitarios Regional de Málaga y Virgen de la Victoria, Spain

HIGHLIGHTS

- Anxiety-like behavior predicts cocaine-conditioned place preference (CPP) in mice.
- Cocaine-induced CPP was not predicted by other behavioral measures.
- High CPP-expressing mice had more neurons in the basolateral amygdala (BLA).
- Anxiety may increase vulnerability for cocaine, with the BLA as a substrate.

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ABSTRACT

The identification of behavioral traits that could predict an individual's susceptibility to engage in cocaine addiction is relevant for understanding and preventing this disorder, but investigations of cocaine addicts rarely allow us to determinate whether their behavioral attributes are a cause or a consequence of drug use. To study the behaviors that predict cocaine vulnerability, male C57BL/6J mice were examined in a battery of tests (the elevated plus maze, hole-board, novelty preference in the Y-Maze, episodic-like object recognition and forced swimming) prior to training in a cocaine-conditioned place preference (CPP) paradigm to assess the reinforcing value of the drug. In a second study, the anatomical basis of high and low CPP in the mouse brain was investigated by studying the number of neurons (neuronal nuclei-positive) in two addiction-related limbic regions (the medial prefrontal cortex and the basolateral amygdala) and the number of dopaminergic neurons (tyrosine hydroxylase-positive) in the ventral tegmental area by immunohistochemistry and stereology. Correlational analyses revealed that CPP behavior was successfully predicted by anxiety-like measures in the elevated plus maze (i.e., the more anxious mice showed more preference for the cocaine-paired compartment) but not by the other behaviors analyzed. In addition, increased numbers of neurons were found in the basolateral amygdala of the high CPP mice, a key brain center for anxiety and fear responses. The results support the theory that anxiety is a relevant factor for cocaine vulnerability, and the basolateral amygdala is a potential neurobiological substrate where both anxiety and cocaine vulnerability could overlap.

* Corresponding author at: Departamento de Psicobiología y Metodología de las CC, Facultad de Psicología, Universidad de Málaga, Campus de Teatinos S/N, 29071 Málaga, Spain. Fax: +34 952 132 621.
** Corresponding author at: Laboratorio de Medicina Regenerativa, Hospital Regional Universitario de Málaga, Pabellón de Gobierno (sótano), Avenida Carlos Haya 82, 29010 Málaga, Spain. Fax: +34 952 614 102.
E-mail addresses: luis@uma.es (L.J. Santín), estela.castilla@ibima.eu (E. Castilla-Ortega).

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1. Introduction

Cocaine addiction is a chronic brain disease with an important global burden, as it requires complicated treatment and has many health and socioeconomic implications. Cocaine is used by 14–21 million people worldwide (prevalence of ~0.4%), most frequently in North and South America, Oceania and Western and Central Europe [1], where 4.2% of Europeans (15–64 years old) have used cocaine in their lifetime [2]. However, only a subset of cocaine users will become addicted. While some individuals lose control over their cocaine intake and eventually become addicts, others use the drug without developing abusive tendencies or dependence. In fact, it is estimated that only 20.9% of cocaine users become dependent on cocaine at some point in their lives [3].

Key individual differences account for both the acquisition and maintenance of drug-related behaviors. Many biological, socioeconomic and psychological variables modulate the drug's perceived reinforcing value or a person's control over its use, thereby influencing drug addiction vulnerability [3,4]. Regarding the psychological vulnerability factors, studies have identified several stable, and likely heritable, behavioral characteristics or 'personality traits' that may increase the risk of cocaine addiction. A dimension involving active searching for sensations and new experiences ('sensation seeking' or 'novelty seeking') is often elevated in cocaine addicts compared to controls and is related to the severity of cocaine use [5–7]. Moreover, cocaine addiction usually involves increased impulsivity and willingness to take risks, cognitive deficits and an altered emotional status that frequently corresponds with high anxiety [8,9], which are expressed as elevated psychiatric comorbidities, such as anxiety and mood disorders [10,11]. An underlying assumption is that these behavioral attributes reflect the abnormal function of key brain areas that are also part of the addiction circuitry and that respond to drug abuse in a maladaptive way, triggering addiction. In this regard, magnetic resonance studies have associated cocaine addiction with macrostructural abnormalities (i.e., volume alterations) in the limbic brain regions that are involved in motivational, cognitive and emotional behavior, such as the frontal cortices and temporal lobe structures, including the amygdala but usually not the hippocampus [12–19].

Nevertheless, it is difficult for human studies to elucidate whether the maladaptive behavioral traits and the neurobiological hallmarks found in addicts actually predispose individuals to cocaine addiction or if they are a consequence of the neuroadaptations induced by prolonged drug intake. Research on this topic benefits from animal models, as drug administration can be controlled allowing to examine their behaviors prior to drug exposure. It is consistently reported that mice or rats that exhibit an increased response to novelty, have elevated impulsivity or high anxiety-like behavior, display increased cocaine-seeking behavior when exposed to self-administration or conditioned place preference paradigms (novelty: [20–23]; impulsivity: [24,25]; anxiety: [26–29]). However, while rodents' performances in certain tasks could emulate stable behavioral traits similar to those in humans, the meaning of the rodents' behaviors is often confusing because activity/exploration, emotion and cognition cannot easily be distinguished in animal tests. For example, behaviors that are used as reliable measures for the novelty response (i.e., locomotion or hole exploration) can be influenced by the emotional state experienced by the animal, while the most frequently used anxiety-like measures (i.e., time spent exploring the unprotected zones of a maze) also involve a locomotor/exploratory response (reviewed in [30]). Indeed, this issue may explain some of the complex results on rodents' addiction-vulnerability behaviors in the literature [23,29,31]. On the other hand, many studies usually fail to analyze the neurobiological basis of vulnerability in animals.

In this experiment, the mice underwent a battery of tests for a wide range of behavioral domains: novelty-induced exploration and preference for novel contexts, emotion (unconditioned anxiety and despair-like behavior) and hippocampal-dependent memory. Subsequently, the cocaine-seeking behavior was tested in the conditioned place preference (CPP) paradigm, a widely used test to study the reinforcing value of drugs [32]. Correlational approaches were employed to determine whether a behavioral dimension underlying the performance of the mice in the exploratory, emotional or cognitive tests could predict cocaine-seeking behaviors. In addition, animals with high or low place preference were studied for structural differences (the total number of neurons) in the medial prefrontal cortex (mPFC) and the basolateral amygdala (BLA), two key limbic brain areas that may support the link between addiction-vulnerability behavior and cocaine-seeking behavior.

2. Materials and methods

2.1. Animals

The male C57BL/6J mice were acquired from Janvier (Le Genest-St-Isle, France) and arrived at the animal facility when they were 11 weeks of age. After one week of acclimation, the mice were individually housed in standard laboratory cages with nesting material and were handled daily (5 min/day) for five days. The mice were maintained on a 12 h light/dark cycle (lights on at 8:00 a.m.) with water and food provided ad libitum.

The experimental procedures were performed in accordance with the European (Directive 2010/63/UE) and Spanish regulations (Real Decreto 53/20130 and Ley 32/2007) for animal research. The protocol was approved by the Comité Ético de Experimentación Animal of the University of Málaga (permit number: CEUMA n° 8-2014-A). All surgery (i.e., intravascular perfusion) was performed under sodium pentobarbital anesthesia (200 mg/kg) and all efforts were made to minimize the animal's suffering.

2.2. Experiment I: behaviors that predict conditioned place preference

Sixteen mice were used for this experiment and began the behavioral protocol at 13 weeks of age. Behavioral testing was performed between 8:00 a.m. and 3:00 p.m. in a noise-isolated room illuminated by 200 lux. The mice were habituated to the room for at least 20 min before the assessment began. After each session, the apparatuses were carefully cleaned with a solution of 70% alcohol to remove the odor cues. The sessions were recorded with a digital camera and analyzed with the Ethovision XT9 software (Noldus, Waninghen, The Netherlands) to determine the spatiotemporal parameters. Stereotypic behaviors were observed by a trained experimenter using the Ethovision's Manual Score module.

2.2.1. Exploratory, emotional and cognitive tests

From Days 1 to 5, the mice were submitted to a behavioral test battery to assess activity/exploration, emotion and cognition, based on previously reported methods [30,33–37]. Because the mice would progressively become habituated to the behavioral test, the tasks to assess exploration and unconditioned anxiety were performed on the first days, when the testing environment could elicit both novelty and aversion; while a highly stressful task (i.e., the forced swimming test) was administered last to avoid its potential deleterious influence on the subsequent behavioral measures. Thus, the behavioral assessment was performed in the following sequence: the elevated plus maze test (EPM, Day 1) and the hole-board test (HB, Day 2) for exploratory and anxiety-like behavior, the Y-Maze test for novelty preference (Day 3), the episodic-like object recognition memory test (Day 4) and the forced swimming test