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AMPHETAMINE SENSITIZATION IS ACCOMPANIED BY AN INCREASE IN PRELIMBIC CORTEX ACTIVITY

M. I. AGUILAR-RIVERA, [b†] **J. P. CASANOVA,** [b†] **R. I. GATICA,** [a,b] **G. J. QUIRK** [c,d] **AND J. A. FUENTEALBA** [a,b*]

[a] *Department of Pharmacy, Faculty of Chemistry, Pontificia Universidad Católica de Chile, Santiago, Chile*

[b] *Millennium Science Nucleus in Stress and Addiction, Pontificia Universidad Católica de Chile, Santiago, Chile*

[c] *Department of Psychiatry, University of Puerto Rico School of Medicine, San Juan, Puerto Rico*

[d] *Department of Anatomy and Neurobiology, University of Puerto Rico School of Medicine, San Juan, Puerto Rico*

Abstract—Drug addiction is associated with dysfunction in the medial prefrontal cortex (mPFC). However, the modifications of neuronal activity in mPFC underlying the reinforcing properties of addictive drugs are still unclear. Here we carried out single-unit recording experiments to study the neuronal activity in the prelimbic (PL) cortex of anesthetized rats, after expression of locomotor sensitization to amphetamine. In control rats, an acute injection of amphetamine induced mainly an inhibitory effect on firing rate (FR) and this response was negatively correlated with the basal FR. Sensitized rats showed a higher proportion of excited neurons and the response to amphetamine was independent of basal FR. Moreover, in control rats, acute amphetamine decreased burst rate, whereas in sensitized rats acute amphetamine increased burst rate. These findings indicate that amphetamine sensitization renders mPFC neurons hyperexcitable. Taken together, these data support the hypothesis that early withdrawal is associated with an increase in the activity of the mPFC, which could strengthen the PL–Nucleus Accumbens connection, thus facilitating amphetamine-induced locomotor sensitization.

Key words: medial prefrontal cortex, amphetamine, locomotor sensitization, single-unit recording.

INTRODUCTION

Drug addiction is a cluster of cognitive, behavioral and physiological symptoms that reveal the compulsive use of the drug despite adverse consequences of its consumption (American Psychiatric Association, 2000). It has been proposed that chronic exposure to drugs of abuse induces dysfunction in the medial prefrontal cortex (mPFC), associated with a failure in inhibitory control that may contribute to compulsive drug-seeking behavior (Jentsch and Taylor, 1999). Clinical studies using functional neuroimaging have highlighted that drug abusers show an increased metabolic activity in the mPFC associated with early withdrawal and drug expectation (craving), and a decreased mPFC basal activity associated with protracted withdrawal (Goldstein and Volkow, 2002, 2011). These results suggest that time-depending modifications in the mPFC take place after repeated use of drugs of abuse.

In this regard, pre-clinical studies have shown consistent evidence of neuroplastic changes in the mPFC induced by repeated exposure to cocaine and amphetamine (Steketee, 2003; Van den Oever et al., 2010; Steketee and Kalivas, 2011). A persistent increase in excitability of pyramidal neurons of the mPFC is observed after short and long-term withdrawal following repeated administration of cocaine (Nasif et al., 2005). In line with this finding, a reduction in the $GABA_B$-dependent inhibition of pyramidal neurons from the prelimbic (PL) cortex that accompanied cocaine locomotor sensitization has been reported (Hearing et al., 2013). Moreover, Kasanetz et al. (2013) have identified synaptic markers in the PL cortex that may predict vulnerability to drug addiction. Specifically, rats that develop an addiction-like behavior after cocaine self-administration show a selective attenuation of presynaptic long-term depression and a concomitant postsynaptic increase in AMPA/NMDA ratio (Kasanetz et al., 2013). These results suggest that increased excitatory tone in PL neurons may underlie drug-seeking behavior. Altered mPFC functioning contributes to maintain drug-seeking behavior, but the mechanism underlying this phenomenon is still poorly understood. In fact, experiments using optogenetics show that both silencing (Stefanik et al., 2013) and activating (Chen et al., 2013) PL neurons decrease cocaine-seeking behavior. These data suggest a complex mechanism of the mPFC network underlying behavioral inhibitory control.

The incentive sensitization theory of drug addiction (Robinson and Berridge, 1993) states that repeated exposure to addictive drugs renders brain circuits, that regulate the attribution of emotional prominence to stimuli, hypersensitive (Robinson and Berridge, 2008). This com-

*Correspondence to: J. A. Fuentealba, Department of Pharmacy, Faculty of Chemistry, Pontificia Universidad Católica de Chile, Santiago, Chile. Tel: +56-2-2354-5908; fax: +56-2-2354-4744.
E-mail address: jfuentea@uc.cl (J. A. Fuentealba).
† Equal contribution.
Abbreviations: ANOVA, analysis of variance; DA, dopamine; FR, firing rate; ISI, interspike interval; mPFC, medial prefrontal cortex; PL, prelimbic.

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plex emotional sensitization is indirectly evidenced by locomotor sensitization (Robinson and Berridge, 2008), which is a valid pre-clinical model to explore the neural basis of addiction (Steketee and Kalivas, 2011). Repeated exposure to amphetamine induces locomotor sensitization (Casanova et al., 2013) and a long-lasting increase in the length and density of dendritic spines in pyramidal neurons of the mPFC (Robinson and Kolb, 1997). It is well established that morphological and neurochemical changes in mPFC contribute to persistence in the reinforcing properties of psychostimulants (Steketee, 2003), but few studies have addressed how these changes modify neuronal activity of PL neurons. Interestingly, both, an increased (Gulley and Stanis, 2010) and a decreased (Homayoun and Moghaddam, 2006) responsiveness in firing rate (FR) to amphetamine was observed after its repeated administration. However, in these studies the behavioral changes were either not measured (Homayoun and Moghaddam, 2006) or it is not clear whether all animals did show behavioral sensitization (Gulley and Stanis, 2010).

Two stages have been identified in behavioral sensitization after repeated amphetamine administration: development and expression. Development involves transient neuronal modifications leading to enduring changes responsible for enhanced locomotor activity observed during the expression of sensitization (Pierce and Kalivas, 1997). For example, we previously showed consistent modifications in mPFC dopamine (DA) extracellular levels in rats showing development and expression of sensitization (Casanova et al., 2013). Given that the activation of DA receptors modifies the basal firing of PL neurons (Trantham-Davidson et al., 2004), we propose that changes in PL activity that accompanies expression of sensitization would take place in rats showing a robust development of locomotor sensitization to amphetamine. Here we investigated the effect of an acute dose of amphetamine on the electrical activity of PL neurons of anesthetized rats. Single-unit recording experiments were performed in rats showing development of sensitization, twenty-four hours after the expression of locomotor amphetamine sensitization was confirmed.

EXPERIMENTAL PROCEDURES

Animals

Adult male Sprague–Dawley rats (weight: 300–340 g) were raised in the Animal Care Facility of the Biological Science, Pontificia Universidad Católica de Chile, under the advice of a veterinarian. Rats were maintained during drug treatment in the Animal Care Facility of the Department of Pharmacy, Pontificia Universidad Católica de Chile, following the instruction of a supervisory protocol given by the veterinarian. Rats were housed in a colony room in groups of three per cage and were kept at room temperature of 20 ± 3 °C on a 12-h light/dark cycle (light on at 7 AM, Eastern Standard Time) with a food regimen and water *ad libitum*. All procedures were in strict accordance with the guidelines published in the "NIH Guide for the Care and Use of Laboratory Animals (8th ed.) and the principles presented in the "Guidelines for the Use of Animals in Neuroscience Research" by the Society for Neuroscience. Rats were handled for one week before starting the experiments.

Amphetamine sensitization schedule

The process of sensitization was adapted from the amphetamine sensitization schedule described by Hedou et al. (2001). Rats were placed in a test cage (15 cm × 47 cm × 26 cm) equipped with a metal mesh floor and two pairs of infrared lights arranged longitudinally, separated by 25 cm and 5 cm above ground. Crossovers in the test cage were monitored using a counting device programed to count one crossover when both infrared lights were interrupted consecutively. Once in the test cage, rats were habituated for 20 min to attenuate normal exploratory activity. Then, rats were injected with D-amphetamine 1.5 mg/kg i.p. (Laboratorio Chile, Santiago, Chile) or an equivalent volume of saline (control) and horizontal locomotor activity was measured immediately during 50 min. This procedure was carried out at 11 am, once a day for five consecutive days (days 1–5). After four days of abstinence (days 6–9) a challenge dose of amphetamine (1.5 mg/kg i.p) was injected to both groups and locomotor activity was measured to assess the expression of locomotor sensitization (day 10) (Fig. 1). We consider locomotor sensitization when the

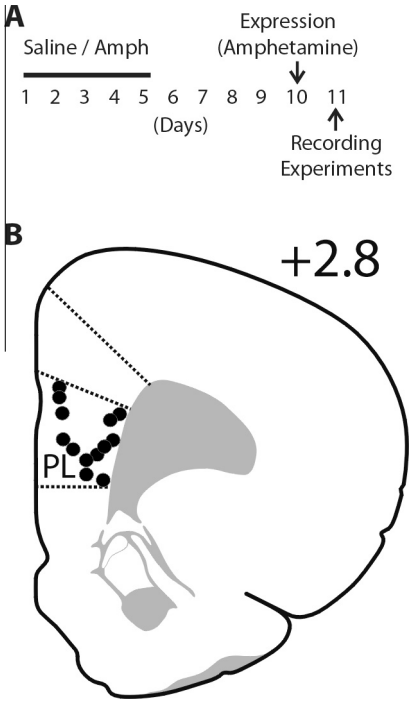

Fig. 1. Schedule of amphetamine sensitization. (A) Protocol used to induce amphetamine sensitization. Rats were injected once daily with amphetamine 1.5 mg/kg i.p. or equivalent volume of saline for five consecutive days. After 4 days of withdrawal (day 10), all rats were injected with 1.5 mg/kg i.p. of amphetamine. Single-unit recording experiments were performed 24 h after amphetamine challenge (day 11). (B) Coronal drawing shows location of recording electrodes [Bregma +2.8 mm; adapted from Paxinos and Watson (2005)].