Neurobiology of Learning and Memory 138 (2017) 281–290

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Persistent strengthening of the prefrontal cortex – nucleus accumbens pathway during incubation of cocaine-seeking behavior

Catarina Luís [a,b], Nazzareno Cannella [b], Rainer Spanagel [b], Georg Köhr [a,b,c,*]

[a] Physiology of Neural Networks, Central Institute of Mental Health, Medical Faculty Mannheim/Heidelberg University, Germany
[b] Institute of Psychopharmacology, Central Institute of Mental Health, Medical Faculty Mannheim/Heidelberg University, Germany
[c] Department of Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim/Heidelberg University, Germany

ARTICLE INFO

Article history:
Received 4 July 2016
Revised 9 September 2016
Accepted 4 October 2016
Available online 5 October 2016

Keywords:
Cocaine self-administration
Reinstatement
In vivo electrophysiology
Field potential
Paired-pulse ratio
Presynaptic glutamate

ABSTRACT

High rates of relapse after prolonged abstinence are often triggered by exposure to drug-associated cues that induce drug craving. Incubation of drug craving is a phenomenon that consists of time-dependent increases in cue-induced drug craving during withdrawal. Plasticity mechanisms in the nucleus accumbens (NAc) underlie drug-seeking responses and involve changes in excitatory synaptic transmission's efficacy. In particular, the prefrontal cortex (PFC) glutamatergic input to the NAc core has been well characterized regarding cocaine-evoked plasticity following non-contingent versus contingent exposure to cocaine or alternatively after protracted abstinence. Still, the synaptic strength during the course of withdrawal compared to drug-naïve condition is unknown, since electrophysiological characterizations are mainly performed in brain slices or focus on distinct time points during cocaine-evoked plasticity *in vivo*. Here we used an incubation paradigm, in which rats had extended accessed to cocaine self-administration, and underwent cue-induced reinstatement at withdrawal day 1 and 30. Longitudinal *in vivo* field potential recordings in awake rats showed that chronic contingent exposure to cocaine strengthened the prelimbic PFC to NAc core pathway when compared to pre-cocaine condition. This strengthening was associated with decreased paired-pulse ratios (PPR), indicative of presynaptic enhancement of glutamate release, which persisted throughout withdrawal. Moreover, both field potential increase and PPR reduction after chronic cocaine exposure correlated with the number of cocaine infusions received during training. The present results together with previous findings of withdrawal-dependent postsynaptic enhancement of the PFC-NAc core pathway, suggest an additional presynaptic strengthening that is initiated during self-administration and maintained throughout abstinence in drug-seeking rats. These cocaine-driven neuroadaptations may provide a neural substrate for maladaptive processing of cues that can ultimately trigger craving and relapse.

1. Introduction

Enduring vulnerability to relapse that persists long after cessation of drug intake is one of the most important hallmarks of addiction. Recurrent relapse and craving, often triggered by cues previously associated with drug use, are perhaps the most clinically relevant and challenging features to manage in patients recovering from addictive disorders. In fact, incubation of drug craving, an extensively studied phenomenon, describes a gradual increase in cue-induced craving in humans (Bedi et al., 2011; Gawin & Kleber, 1986; Li et al., 2015) and drug-seeking in animals (Grimm, Hope, Wise, & Shaham, 2001; Li, Caprioli, & Marchant, 2015) during abstinence, which can persist over several months following halting drug use (Lu, Grimm, Dempsey, & Shaham, 2004). This augmented drive to seek and use drugs is thought to be rooted in long lasting drug-induced neuroadaptations, specifically in excitatory synaptic transmission's efficacy (Huang, Schluter, & Dong, 2015; Lüscher, 2016; Wolf, 2016) as well as intrinsic plasticity (Kourrich, Calu, & Bonci, 2015).

Drug-dependent alterations of the glutamatergic system have been demonstrated to play a major role in mediating incubation of drug-seeking as well as in driving abnormal learning associated with progressively greater behavioral emphasis towards the drug and drug associated cues at the expense of natural rewards (Kalivas, Volkow, & Seamans, 2005). In particular, the prefrontal cortex (PFC) to nucleus accumbens (NAc) pathway emerges as instrumental in regulating drug-seeking responses (Bossert, Marchant,

* Corresponding author at: Physiology of Neural Networks, Psychiatry/Psychopharmacology, Central Institute of Mental Health (ZI), J5, D-68159 Mannheim, Germany.
E-mail address: georg.koehr@zi-mannheim.de (G. Köhr).

http://dx.doi.org/10.1016/j.nlm.2016.10.003
1074-7427/

Calu, & Shaham, 2013; Li, Caprioli et al., 2015). Thus, activation of the PFC projection to the NAc core was shown to mediate drug-seeking responses which were associated with elevations in NAc glutamate (McFarland, Lapish, & Kalivas, 2003). Similarly, in heroin-seeking animals glutamate release from PFC projections to the NAc core is required for cue-induced reinstatement (LaLumiere & Kalivas, 2008). Consistently, optogenetic weakening of PFC afferents onto the NAc core was found to diminish cue-induced reinstatement of cocaine-seeking behavior (Stefanik et al., 2013). Furthermore, both pharmacological and optogenetic inhibition of the ventral mPFC decreased incubated cocaine-seeking behavior (Koya et al., 2009; Ma et al., 2014). This effect was recapitulated by reversible inactivation of glutamatergic transmission in the NAc core (LaLumiere & Kalivas, 2008; McFarland & Kalivas, 2001), which also results in attenuated reinstatement. Increased glutamatergic drive in the accumbens core may also occur during long-lasting abstinence. In this regard, *in vivo* unit recordings conducted at later stages of withdrawal have shown an increase in NAc core neurons responsiveness to cocaine associated cues when compared to early withdrawal (Hollander & Carelli, 2007). This increased responsiveness of NAc core neurons may be due to an increased synaptic expression of calcium permeable AMPA receptors (Conrad et al., 2008; Loweth, Tseng, & Wolf, 2014) as well as calcium impermeable AMPA receptors (Ma et al., 2014). In general, multiple changes in excitatory transmission that also take place in NAc shell neurons pre- and postsynaptically (Mameli et al., 2009; Suska, Lee, Huang, Dong, & Schlüter, 2013; Ma et al., 2014) have been identified that contribute to a drug-seeking response.

Here, we sought to identify electrophysiological neuroadaptations associated with cocaine self-administration and withdrawal. We conducted a longitudinal study that allowed us to monitor PFC-NAc core synaptic transmission, via *in vivo* field potential recordings, over time within individuals. Our *in vivo* results substantiate previous observations of a presynaptic enhancement of the PFC-NAc pathway during cocaine self-administration and protracted withdrawal that may contribute to the development of incubation.

2. Material and methods

2.1. Animal housing and surgery

All experiments were conducted at the Central Institute of Mental Health in Mannheim (Germany). Experimental procedures were in accordance with the Directive 2010/63/EU guidelines for care and use of laboratory animals, and were approved by the local animal care committee (G-273/12; Regierungspräsidium Karlsruhe, Germany). Eleven male Sprague-Dawley rats (Charles River, Germany), seven week old at their arrival, were single housed under 12 h dark/light reverse cycle in a temperature (22 ± 1 °C) and humidity (60 ± 5%) controlled room. Subjects were given access to food and water *ad libitum* throughout the experiment. Two weeks after arrival rats were implanted with a catheter (Micro-Renathane®) inserted in the right jugular vein under isoflurane anesthesia. Catheters were flushed daily with a heparinized solution (100 I.U./ml) containing enrofloxacin (1 mg/ml, Baytril®). Self-made bipolar tungsten electrodes (52 µm, California Fine Wire, Grover Beach, California, USA) were chronically implanted in the prelimbic PFC for stimulation (AP +3.0, ML +0.6, DV −3.3 mm from brain surface) and in the NAc core for recording (AP +1.8, ML +1.3 to 1.5, DV −5.5 to −6.5 mm from brain surface), according to the Paxinos and Watson atlas. The final position of the electrodes was determined by online monitoring evoked field potentials, depth profiles and input-output (IO) curves under ketamine/xylazine anesthesia (65/14 mg/kg; Fig. 1A). Animals were given 7–9 days recovery before cocaine self-administration (CSA) sessions and electrophysiological recordings began.

2.2. Drugs

Cocaine-HCl (Sigma-Aldrich, Germany) was dissolved in sterile saline and self-administered by the subjects via intravenous (i.v.) route.

2.3. Self-administration apparatus

Self-administration chambers (40 cm long × 30 cm wide × 52 cm high; Fig. 1B) were located in sound-attenuating cubicles equipped with exhaust fans to assure air renewal. Two poke holes were on opposite walls of the chambers, 5 cm above the grid floor. When rats poked their snout in the holes, breaking an infrared beam, their instrumental responding was recorded. One hole was associated with cocaine delivery and designated as the active hole, while the other was designated as the inactive hole and served as control. A white cue light was located 9.5 cm above the active hole, and a blue light was on the left side of the opposite wall 33 cm above the grid floor. A speaker allowing presentation of

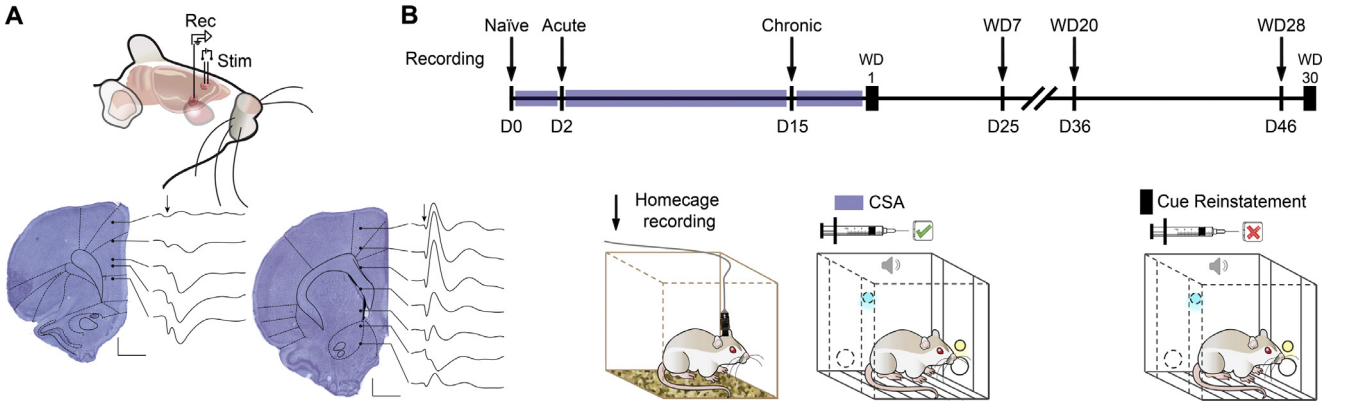

Fig. 1. Field potential responses and experimental timeline illustrating cocaine self-administration (CSA) paradigm. (A) Scheme showing stimulation electrode in the PFC (Stim) and recording electrode in the NAc core (Rec) of a rat. The lower panel depicts depth profiles, which were acquired while lowering the stimulation electrode to the prelimbic PFC (left) and maintaining the recording electrode stationary in the NAc core (DV 5.5–6.5); or while lowering the recording electrode to the NAc core (right) and maintaining the stimulating electrode in the PFC unchanged (DV ≈3.3). Representative traces are averages of ten consecutive ePSPs (scale bar, 0.5 mV and 25 ms). Time of stimulation is indicated by arrow. (B) Experimental timeline indicating days of home cage recordings at distinct time points (arrow) during both CSA (purple) and 28 days of withdrawal (WD) for cocaine group as well as for control group labeled D0 to D46. The lower panel depicts home cage recording setup, operant chamber during CSA (purple, green check) and during cue-induced reinstatement in the absence of reward (black, red cross), which took place at WD1 and 30.