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Opiate dependence induces network state shifts in the limbic system

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ABSTRACT

Among current theories of addiction, hedonic homeostasis dysregulation predicts that the brain reward systems, particularly the mesolimbic dopamine system, switch from a physiological state to a new "set point." In opiate addiction, evidence show that the dopamine system principal targets, prefrontal cortex (PFC), nucleus accumbens (NAC) and basolateral amygdala complex (BLA) also adapt to repeated drug stimulation. Here we investigated the impact of chronic morphine on the dynamics of the network of these three interconnected structures. For that purpose we performed simultaneous electrophysiological recordings in freely-moving rats subcutaneously implanted with continuous-release morphine pellets. Chronic morphine produced a shift in the network state underpinned by changes in Delta and Gamma oscillations in the LFP of PFC, NAC and BLA, in correlation to behavioral changes. However despite continuous stimulation by the drug, an apparent normalization of the network activity and state occurred after 2 days indicating large scale adaptations. Blockade of μ opioid receptors was nonetheless sufficient to disrupt this acquired new stability in morphine-dependent animals. In line with the homeostatic dysregulation theory of addiction, our study provides original direct evidence that the PFC–NAC–BLA network of the dependent brain is characterized by a *de novo* balance for which the drug of abuse becomes the main contributor.

Introduction

Opiate use (heroin or morphine) induces a fast and powerful dependence and the prevalence of opiate use and abuse has increased in the past few years with a major impact on public health (http://www.who.int/substance_abuse/facts/opiates/en/). Among current theories of addiction, hedonic homeostasis dysregulation predicts that the brain reward systems, particularly the mesolimbic dopaminergic circuit, switch from a physiological state to a new "set point" (also named allostatic state) as dependence develops (Ahmed and Koob, 1998; Koob and Le Moal, 1997). The prefrontal cortex (PFC), nucleus accumbens (NAC) and basolateral amygdala (BLA) are main targets of the dopamine system and represent core structures at the interface of drug-reinforcement and drug and cue-reinstatement circuits which are crucial features of addiction (for review, Le Moal and Koob, 2007). These regions are the site of severe functional adaptations and homeostatic impairments following chronic drug exposure (Christie, 2008; Kalivas, 2009; Luscher and Malenka, 2011).

PFC, NAC and BLA form an interconnected limbic network; in which PFC and BLA are reciprocally connected and both project to the NAC (Cardinal et al., 2002). It is widely accepted that brain functions are distributed processes and that distant structures associated in a functional network interact through oscillatory and phase synchronization of neuronal activity (Fell and Axmacher, 2011). In line with this idea several studies have demonstrated that rhythmic interactions between PFC, NAC and/or BLA in theta (5–10 Hz) and gamma (40–100 Hz) oscillations are central to cognitive functions such as learning and memory (Berke, 2009; Popa et al., 2010; Popescu et al., 2009). In opiate addiction a few pioneer studies have investigated the impact of the drug on oscillatory processes in these structures. In both opiate dependent patients and rats, repeated morphine treatment alters the EEG in the prefrontal cortex which is subject to a marked increase in delta range oscillations (1–4 Hz) correlated with drug intake (Greenwald and Roehrs, 2005; Sun et al., 2006). Surprisingly, while a few studies have documented the effect of psychostimulants on deep structure dynamics, such as the NAC (Berke, 2009) and the hippocampus (Liu et al., 2010), the effect of opiates on NAC and BLA oscillatory activity as well as on the synchronization between PFC, NAC and BLA remains largely unexplored. Such

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functional connectivity has been partially investigated in a recent *in vitro* study which revealed that long-term depression and potentiation were both impaired at the PFC to NAC synapses in rat self-administrating heroin (Shen and Kalivas, 2012). This result shows that functional interactions are modified for at least one node of the PFC, NAC and BLA ensemble; however, to date no study has gathered the experimental conditions required to investigate neuronal dynamics at the whole network level.

In healthy subjects physiological behaviors present distinct network stable states in the forebrain characterized by specific oscillatory and synchronization profiles (Gervasoni et al., 2004). Abnormal oscillatory signatures observed in the PFC under opiates indicate that chronic drug administration is able to challenge local network stability (Greenwald and Roehrs, 2005; Sun et al., 2006). Considering that PFC, NAC and BLA are functionally bound through oscillatory synchronization, this strongly suggests that the dynamics of the entire network could be modified in opiate dependence and give rise to novel drug-related states and equilibrium. In line with this idea and current theories of addiction, we postulated that chronic morphine stimulation impairs PFC–NAC–BLA functional equilibrium by promoting the emergence of a drug related *de novo* network state. While studies so far have focused on single structures, testing this hypothesis requires an original and global approach. For that purpose we used simultaneous electrophysiological recordings in the PFC NAC and BLA in freely-moving rats combined with multivariate analysis methods to characterize network states under physiological conditions and after chronic morphine.

Materials and methods

Animals

Thirteen male Sprague–Dawley rats (Charles River Laboratories, France) were individually housed under an inverted 12-h light/dark cycle (lights off at 8:00 h) at 21 ± 2 °C with food and water available *ad libitum*. Weight varied from 250 to 300 g at the beginning of the study to 300–350 g at the time of the last recording. Surgical and experimental procedures were performed in accordance with the European Community's Council Directive (EU Directive 2010/63/EU86) and the National Institute of Health guide for the care and use of laboratory animals. The present experiment was approved by the local Animal care and Use committee (approval #5012049-A).

Electrode implantation surgery

Rats were implanted with 12 independently moveable tetrodes in PFC (2 tetrodes), NAC (4 tetrodes) and BLA (6 tetrodes). Surgery took place after at least 7 days of daily handling habituation, under full general gas anesthesia (isoflurane 1.5–2%) with local anesthetic (xylocaine 0.5%) at the incision site and under prophylactic antibiotic (ampicillin, 7 mg/kg). The recording electrodes were lowered to position through small craniotomies above each structure. Six stainless-steel skull screws were inserted and the implant was affixed to the skull with super bond and dental acrylic. Two of the skull screws were positioned over the cerebellum approximately 3 mm caudal to lambda and used for animal grounding and electrode referencing. After the surgery animals were injected with carboprofen (2 mg/kg, s.c.) for pain management and allowed to recover from anesthesia before being returned to the animal housing facilities.

Induction of morphine dependence and withdrawal

Dependence was induced by the subcutaneous implantation of two morphine pellets. Compared to repeated injections or intermittent self-administration protocols this approach allows to reach a drug-dependent state without the animal being exposed to repeated withdrawal experiences. Indeed this has been shown to modify neuronal activity and plasticity in our structures of interest (Lucas et al., 2008) and may therefore interfere with the sole effect of chronic morphine analyzed here. Moreover, this model allows the use of naloxone to precipitate and exactly time the onset of withdrawal. Thus after 5–7 days of recovery from the first surgery, animals were implanted with subcutaneous pellets. The experimental group ($n = 8$) received two morphine pellets (2 × 75 mg of morphine base; NIDA, USA) and a control group received two placebo pellets ($n = 5$). Under general anesthesia (isoflurane 3%) a small incision was made on the animal's back and two pellets were placed in the lumbar region, one on each side of the medial line. Two stitches were set at the incision site before the animal was returned to the housing facility.

Under morphine, pellet drug dependence is classically obtained after 24 hours and lasts for at least 12 days (Gold et al., 1994). Behavioral and electrophysiological activities were monitored in drug free conditions (Baseline) and for four consecutive days starting 24 hours after pellets implantation (days 1, 2, 3 and 4). Morphine and Placebo groups were then both subjected to an injection of saline (s.c.) on day 5 and of the μ opioid receptor antagonist naloxone on day 6 (Sigma, 15 μg/kg s.c.) and were monitored 24 h after that last injection (day 7).

Recordings

For all electrophysiological and behavioral recordings animals were placed for 20 minutes in a cylindrical box. Behavior was monitored with a camera placed over the box and connected to a Cineplex video tracking system (Plexon, TX, USA). In the same sessions we recorded single unit activity and LFP in the PFC, the NAC and the BLA simultaneously using Multichannel Acquisition Processor (MCP, Plexon). The wide band signal (0.1–9000 Hz) collected by the electrodes was pre-amplified (20×) and amplified (50×) before being digitized (40,000 Hz sampling rate for single unit activity and 1000 Hz for LFP) and stored for further analysis. Single units were manually sorted and cluster isolation was tested for significance in Offline sorter (Plexon). Units were then classified as putative projection neurons or interneurons (Fig. S1) using combined spike width and firing rate methods as previously described (Bartho et al., 2004; Berke et al., 2004; Dejean et al., 2012).

Behavioral data analysis

All analyses were performed while the animals were awake. All time intervals during which the animals were sleeping (i.e. displaying immobility with closed eyes) were filtered out and excluded from further analysis. Morphine pellet implantation induces characteristic episodes of a stupor state during which animals display a lack of responsiveness, immobility, a prone position and exophthalmos. These events were monitored offline by carefully inspecting video recordings of experimental sessions and isolated in specific time intervals for stupor related electrophysiological signals to be further analyzed separately for alert behavior epochs.

In morphine-treated rats the behavioral correlates of a 15 μg/kg injection of the μ opioid receptor antagonist naloxone are typical mild drug withdrawal signs, and this dose has no significant effect in placebo controls (Frenois et al., 2002). In the present study we focused on three withdrawal-related parameters that are enhanced defecation, wet dog shakes and teeth grinding. The occurrence of those signs where analyzed offline by a visual inspection of the video recording and an additional investigation of the presence of mechanical artifacts on electrophysiological traces. Indeed both wet dog shakes and teeth grinding presented a characteristic discrete noise signature that allowed us both to confirm the observations derived from video recording inspection and to isolate and filter out those events before further analysis of the signal. Defecation, wet dog shake and teeth grinding were quantified as the number of episodes per 20 minute session and were analyzed in placebo and morphine animals in three different