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ORIGINAL ARTICLE

Prefrontal synaptic markers of cocaine addiction-like behavior in rats

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Defining the drug-induced neuroadaptations specifically associated with the behavioral manifestation of addiction is a daunting task. To address this issue, we used a behavioral model that differentiates rats controlling their drug use (Non-Addict-like) from rats undergoing transition to addiction (Addict-like). Dysfunctions in prefrontal cortex (PFC) synaptic circuits are thought to be responsible for the loss of control over drug taking that characterizes addicted individuals. Here, we studied the synaptic alterations in prelimbic PFC (pPFC) circuits associated with transition to addiction. We discovered that some of the changes induced by cocaine self-administration (SA), such as the impairment of the endocannabinoid-mediated long-term synaptic depression (eCB-LTD) was similarly abolished in Non-Addict- and Addict-like rats and thus unrelated to transition to addiction. In contrast, metabotropic glutamate receptor 2/3-mediated LTD (mGluR2/3-LTD) was specifically suppressed in Addict-like rats, which also show a concomitant postsynaptic plasticity expressed as a change in the relative contribution of AMPAR and NMDAR to basal glutamate-mediated synaptic transmission. Addiction-associated synaptic alterations in the pPFC were not fully developed at early stages of cocaine SA, when addiction-like behaviors are still absent, suggesting that pathological behaviors appear once the pPFC is compromised. These data identify specific synaptic impairments in the pPFC associated with addiction and support the idea that alterations of synaptic plasticity are core markers of drug dependence.

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INTRODUCTION

Addiction is a relapsing disorder characterized by compulsive drug seeking and loss of control over drug intake. The transition to addiction, that is, the shift from controlled drug use to addiction is observed only in a restricted number of drug users after prolonged periods of drug consumption.[1] Although a large number of neuroadaptations induced by drugs of abuse have been described,[2–6] those specifically associated with the transition to addiction in vulnerable individuals remain largely unknown.[1,7]

Using an animal model that after a prolonged period of drug intake discriminates rats showing an addiction-like behavior from rats that maintain a controlled drug use,[8–10] we recently evaluated changes in synaptic plasticity during the transition to addiction.[11] Activity-dependent synaptic plasticity is a major cellular mechanism that mediates the refinement of neuronal circuits necessary to adapt behavior to an ever changing environment and has been proposed to be an important factor leading to compulsive drug use.[12] Specifically, we found that transition to addiction is associated with a form of *anaplasticity* in Addict-like rats, that is, they seem unable to counteract drug-induced impairments in synaptic plasticity that initially occur in all drug-exposed individuals. Thus, a major form of synaptic plasticity called long-term depression (LTD) is suppressed in the nucleus accumbens (NAC) of all subjects[11] during early phases of cocaine self-administration (SA), that is, before the development of addiction-like behavior. However, LTD is progressively recovered in rats that maintain a controlled drug intake (Non-Addict-like rats), but remains disrupted in rats undergoing transition to addiction.[11]

These results have opened a completely unforeseen insight in the pathophysiological mechanism of the vulnerability to addiction. However, while the NAC is crucial in mediating drug reward and drug-seeking behavior, the ability of a subject to control drug intake implicates higher cortical executive brain areas.[13–15] As a consequence, the full understanding of the pathophysiological underpinnings of vulnerability to drugs requires integrating the interplay of drug-induced adaptations between ventrostriatal and cortical, executive-related, brain structures.

To identify potential cortical neuroadaptations specifically associated with the transition to addiction, we have chosen to study synaptic plasticity in the prefrontal cortex (PFC). Indeed, numerous experimental evidence suggest that a decreased executive control over drug-related behaviors may be associated with abnormal activity in the PFC. Human imaging studies in addicts and recording in animals using the cocaine SA paradigm have shown a marked activation of the PFC during the process of drug-related information.[14,16,17] Moreover, cocaine SA alters the morphology of dendrites and dendritic spines of PFC pyramidal cells.[18] Despite this, the synaptic modifications that may compromise PFC function in addiction-like behaving individuals were still not identified.

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Our goal was to identify cortical neuroadaptations that may have a role in transition to addiction. To address this issue, we used the behavioral model cited above that differentiates rats keeping control on their drug use (Non-Addict-like) from rats undergoing transition to addiction (Addict-like), despite equal drug exposure and consumption. We focused on the prelimbic area of the medial PFC (pPFC), a region proposed to control drug seeking.[19–21] Synaptic markers in the pPFC were studied in rats from early to late cocaine use, that is, before and after addiction-like behavior is expressed in vulnerable individuals.

MATERIALS AND METHODS

Subjects

Male Sprague-Dawley rats (280–300 g) were used. Rats were single housed under a 12-h reverse dark/light cycle (on 8 p.m.; off 8 a.m.). Temperature (22 ± 1 °C) and humidity ($60 \pm 5\%$) were also controlled.

Drugs

Cocaine HCl (Coopération Pharmaceutique Française, Bordeaux, France) and Gentamicin (Gentalline®, MSD, France) were dissolved in 0.9% NaCl. Picrotoxin and LY379268 were from SIGMA (St Quentin Fallavier, France) and Tocris (Bristol, UK), respectively. DNQX and AP-5 were from Tocris or Ascent Scientific (Bristol, UK).

SA studies

Surgery. A silastic catheter was implanted in the right jugular vein under ketamine (100 mg kg^{-1}; Imalgéne®, Merial, France) + xylazine (1 mg kg^{-1}; Rompun®, Bayer, France) anesthesia. Rats were allowed to recover for 5–7 days after surgery. Rats received an antibiotic treatment (gentamicin 1 mg kg^{-1} intraperitoneally) during 4 days after surgery. Catheters were flushed daily with a saline solution containing unfractionated heparin (100 IU ml^{-1}).

SA procedures. The SA set-up was previously described in detail.[8,11] The behavioral protocols are extensively detailed in Supplementary Materials and methods.

Basal training protocol

The daily SA session was composed of three drug components (40 min each) separated by two 15 min periods during which responding in the active hole had no scheduled consequences (no drug periods). Drug and no drug periods were signaled with cue lights or illumination of the entire SA box, respectively. During the drug periods, introduction of rat's nose into one hole (active) turned on a white cue light and switched on the infusion pump 1 s later. Nose-pokes in the inactive hole had no scheduled consequences. The self-infusion volume (40 µl, 2 s) contained 0.8 mg kg^{-1} of cocaine. Each infusion was followed by a time-out period of 40 s. The schedule of reinforcement was Fixed Ratio 3 during the first 6 days and increased to Fixed Ratio 5 for the rest of the experiment. Criterion for acquisition of cocaine SA was defined by a stable number of self-infusions over at least three consecutive SA sessions ($\pm 10\%$).

Addiction-like criteria

Cocaine use severity was assessed using three procedures described below and resembling one or more of the seven Diagnostic and Statistical Manual of Mental Disorders IV criteria for addiction.[22]

(i) High motivation for the drug, measured by a progressive ratio schedule of reinforcement. The ratio of responses per infusion was increased after each infusion. The last ratio completed, called the breakpoint, was used to measure motivation for cocaine. (ii) Persistence in drug seeking even if the drug is signaled as unavailable. It was assessed daily by measuring the active responses during the two no drug periods of basal training. (iii) Drug use despite negative consequences, measured by resistance to footshock-induced punishment during cocaine SA. Rats were placed for 40 min in the SA chamber. The cue light signaling drug availability was on. The first response led to the illumination of a green cue light. Rats received an electric footshock (0.2 mA, 1 s) after three additional responses. Then, an electric footshock (0.2 mA, 1 s) and a cocaine infusion (0.8 mg kg^{-1}) associated with its conditioned stimulus was delivered after the fifth response. The green cue light turned off and the schedule was reinitiated at the end of the time-out period. If, within a minute, rats did not complete response requirements leading to shock or shock plus infusion respectively, then the green cue light turned off and the sequence was reinitiated.

Evaluation of cocaine use severity based on the three addiction-like criteria

Establishment of Addict-like and Non-Addict-like groups: a rat was considered positive for an addiction-like behavior when its score for this behavior was in the 35% highest percentile of the distribution. Four groups of rats were then isolated depending on the number of positive criteria met (0crit, 1crit, 2crit and 3crit); 0crit being considered as Non-Addict-like rats and 3crit as Addict-like rats. 1crit and 2crit rats either representing specific stable cocaine 'use-related troubles' or intermediate steps toward addiction (see Belin *et al*.[9] for discussion). A detailed description of the selection procedure is provided in Supplementary Materials and methods.

Addiction score. It was calculated as the algebraic sum of standardized scores of each of the three addiction-like criteria. Standardization consisted in subtracting the mean of the group to each individual score and then dividing this number by the standard deviation.[9]

Establishment of vulnerability to develop an addiction-like behavior. This procedure, previously described[11] and detailed in Supplementary Materials and methods allows a behavioral identification of future 3crit (Addict-like) rats as early as 17 days of SA, based on responding during the no drug periods. In average, 3crit rats tend to progressively increase responding during the no drug periods from session 1 to session 17. This increase is more pronounced from the early to the late minutes of the non-drug periods, as if they were progressively anticipating the next drug period (Supplementary Figure S2). Based on a meta-analysis performed on two previous experiments conducted on 71 rats, four variables characterizing responding during the early no drug periods have been identified which, when combined, allow predicting transition to addiction (Supplementary Materials and methods). Rats are ranked for these four variables. Rats with a score in the twentieth highest percentile of the population are defined as positive for this predictive criterion. Rats with four positive criteria are called Vulnerable, while rats with four negative criteria are called Resistant. The prediction accuracy and potency are described in Supplementary Materials and methods and depicted in Supplementary Figure S3. Active responses during the no drug periods of Resistant and Vulnerable rats identified in the present study are represented in Supplementary Figure S4. As expected, Vulnerable rats progressively increase active responses during the no drug periods from sessions 1 to 17 (Supplementary Figure S4a) while Resistant rats do not. This increase was due to a progressive increase in responses as the 15 min of the no drug periods elapse, as shown for the seventeenth session in Supplementary Figure S4b.

Electrophysiological studies

These methods[11,23] are extensively detailed in Supplementary Materials and methods.

Slice preparation. Rats were anesthetized with a mixture of ketamine (100 mg kg^{-1}) + xylazine (1 mg kg^{-1}) and decapitated. The brain was sliced (300 µm) in the coronal plane while maintained in a sucrose-based physiological solution at 4 °C (in mM: 87 NaCl, 75 sucrose, 25 glucose, 5 KCl, 21 $MgCl_2$, 0.5 $CaCl_2$ and 1.25 NaH_2PO_4). Immediately after cutting, slices were stored for 40 min at 32 °C in an artificial cerebrospinal fluid (ACSF) (in mM): 130 NaCl, 11 glucose, 2.5 KCl, 2.4 $MgCl_2$, 1.2 $CaCl_2$, 23 $NaHCO_3$, 1.2 NaH_2PO_4, and were equilibrated with 95% O_2/5% CO_2. Slices were then stored in ACSF at room temperature until recording. Slices were then placed in the recording chamber and superfused (1.5–2 ml min^{-1})

with ACSF containing picrotoxin (100 μM) to block GABAA receptors. All experiments were performed at ~30 °C. All drugs were added at the final concentration to the superfusion medium.

Recording procedures. Whole-cell patch-clamp recordings were performed from visualized deep layer pyramidal neurons located in the prelimbic area of the PFC. Glass electrodes (resistance 4–6 M) were filled with either Cesium Methane-Sulfonate- or K + Gluconate-based solutions, as follows (in mM): 128 Cesium Methane-Sulfonate or K + Gluconate, 20 NaCl, 1 $MgCl_2$, 1 EGTA, 0.3 $CaCl_2$, 2 Na_2 + ATP, 0.3 Na + GTP, buffered with 10 Hepes, pH 7.3, osmolarity 290–300 mOsm. For field excitatory postsynaptic potential (fEPSP), extracellular recording electrodes were filled with ACSF. To evoke synaptic currents, stimuli (100 μs duration) were delivered at 0.1 Hz with a glass electrode filled with ACSF and placed in layer 2/3 as previously described.[24]

LTD recordings. Endocannabinoid (eCB) mediated LTD (eCB-LTD) was induced stimulating layer 2/3 afferents during 10 min at 10 Hz.[24] metabotropic glutamate receptor 2/3-mediated LTD (mGluR2/3-LTD) of fEPSP was induced by applying the specific mGluR2/3 agonist LY 379268 (100 nM) for 10 min. The magnitude of LTD was estimated from excitatory postsynaptic currents (EPSC)/fEPSP recorded after 25–30 min of inducing LTD.

AMPAR/NMDAR ratio. EPSC were evoked while holding cells at + 40 mV. The AMPAR EPSC was isolated after bath application of the NMDAR antagonist D-2-amino-5-phosphonovaleric acid (100 μM). The NMDAR EPSC was obtained by digital subtraction of the AMPAR EPSC from the dual (AMPAR + NMDAR-mediated) EPSC.

Immunoblotting studies

A detailed description of protein extraction and immunoblotting analysis was previously reported[25,26] and is detailed in Supplementary Materials and methods.

Experimental timeline

First experiment. Two independent groups of animals were used, the first to perform electrophysiological studies (cocaine SA $N = 96$; controls $N = 11$) and the second to carry out western blot analysis (cocaine SA $N = 96$; controls $N = 5$). A progressive ratio was conducted on sessions 40 and resistance to punishment was tested on sessions 45. The mean of the total active responses during the no drug periods of sessions 37–39 were also considered. Addiction severity was evaluated on the bases of the scores of these three addiction-like criteria.

For electrophysiological studies, five additional basal training sessions were performed before the animals started to be tested for the expression of synaptic plasticity. 0crit ($N = 8$), 3crit ($N = 8$) and age-matched control rats ($N = 11$) were alternatively tested (one rat per day). Basal SA training was maintained for all animals until they were tested for electrophysiology. For western blot analysis, 5 3crit and 8 0crit rats were selected.

Second experiment. Rats were trained for cocaine SA according to the basal training protocol during 17 sessions. They were studied for synaptic plasticity after being classified as Vulnerable or Resistant to addiction-like behavior. This procedure was repeated twice in two independent groups of rats ($N = 44$ in each). mGluR2/3-LTD was studied in identified Vulnerable ($N = 6$) and Resistant ($N = 6$) animals from the first group. AMPA/NMDA ratio and eCB-LTD was analyzed in Vulnerable ($N = 6$) and Resistant ($N = 5$) rats from the second group. Rats of matching age and purchase, left undisturbed in the animal house, were used as controls ($N = 14$). To obtain the same time point within groups, the start of the SA sessions of each animal was scheduled appropriately.

Statistics

All values are given as mean ± s.e.m. For SA data, one-way or repeated measures analysis of variance (ANOVA) were used to determine possible group effects and interactions (experimental groups (controls, 3crit, 0crit, Vulnerable, Resistant) were used as between-subject factor). For electrophysiological experiments, N is the number of cells, with at least five animals included in each condition. To determine whether LTD was successfully induced in any given group, a paired Wilcoxon signed rank test was performed comparing the mean EPSC or fEPSP amplitude during baseline and after LTD induction. To evaluate group differences in LTD induction, LTD was expressed by the post-induction response in percent of baseline response. For both LTD and AMPA/NMDA ratio, group differences were assessed with a one-way ANOVA. The Newman–Keuls *post hoc* test was used for pair-wise comparison of means in both SA and electrophysiological experiments. Pearson's correlation analysis was used to assess the relationship between behavior and synaptic parameters. Statistical tests were performed with GraphPad Prism (GraphPad Software, La Jolla, CA, USA) for the electrophysiological data and Statistica 6.0© (StatSoft, Tulsa, OK, USA) for the SA data and the correlation analysis. A critical probability of $P < 0.05$ was applied.

RESULTS

Addiction-like behaviors in rats

After extended daily access to cocaine SA (45 sessions), rats were selected according to their performance in three behaviors that resemble the hallmarks of diagnostic criteria for addiction[1,8,9,11] and that allow discriminating rats that keep control on drug use from rats losing control over drug intake. Rats were grouped based on the number of positive criteria met. Accordingly, rats positive for three addiction-like behaviors (3crit or Addict-like) and rats positive for none of them (0crit or Non-Addict-like) showed clear-cut differences in both the three addiction-like behaviors (see Supplementary Figure S5) and the addiction score resulting from the computation of these three addiction-like behaviors (Figure 1a). In agreement with what we previously reported,[8,11] ~20% of the rats were 3crit while a large proportion of the animals (60%) were 1crit or 0crit. Importantly, Addict-like and Non-Addict-like rats self-administered similar cocaine amounts over the daily basal training SA sessions before they were killed for *ex-vivo* electrophysiology (Table 1).

Cocaine SA suppressed eCB-LTD in both 0crit and 3crit rats

All the electrophysiological experiments, unless otherwise specified, were performed between 50 and 70 days of cocaine SA and the brain slices obtained 24 h after the last SA session.

We first measured retrograde eCB-LTD a form of synaptic plasticity widespread in the central nervous system that exerts a prominent inhibitory control at pyramidal synapses.[24,27] eCB-LTD in pyramidal cells of the pPFC has the potential to be involved in the transition to addiction. Indeed, the eCB system has been implicated in a variety of drug-related behaviors[28–32] and we previously reported how a single non-contingent cocaine injection was sufficient to abolish eCB-LTD at the glutamatergic synapses between the pPFC and the NAC.[33]

In agreement with the idea that cocaine alters eCB-mediated synaptic plasticity, we found that eCB-LTD was impaired in animals that had self-administered cocaine. Unlike controls (75.7 ± 6% of baseline, $P = 0.003$), eCB-LTD was completely suppressed in the pPFC of both 0crit (91.2 ± 10% of baseline, $P = 0.19$) and 3crit rats (100.3 ± 13.1% of baseline, $P = 0.5$) (Figure 1b). Indeed, the experimental groups differed in their response after LTD induction (Group effect, $F(2,18) = 3.95$, $P < 0.05$), with the two cocaine-experienced groups showing a comparable and lower change in synaptic response than controls ($P < 0.05$ for both). In addition, the ability to express eCB-LTD did not correlate with individual addiction scores ($r = 0.36$, $P = 0.26$).

Although cocaine SA induced a clear impairment on eCB-LTD in the pPFC, these data show that the loss of eCB-LTD is a cocaine-induced effect that is not specifically related to transition to addiction.

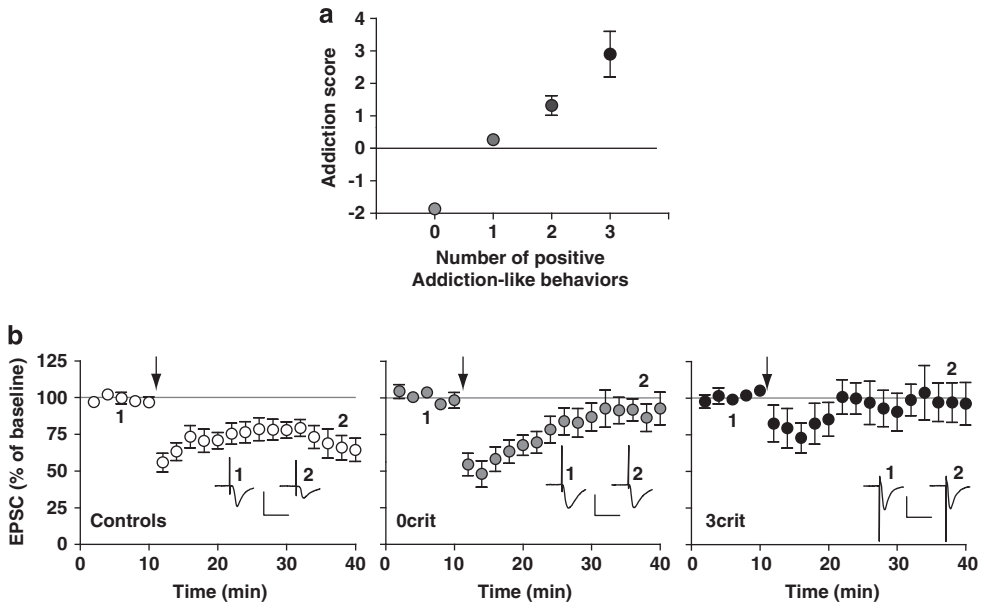

Figure 1. Prolonged cocaine self-administration (SA) abolished endocannabinoid-mediated long-term synaptic depression (eCB-LTD) similarly in Non-Addict-like (0crit) and Addict-like (3crit) rats. (**a**) Addiction scores computed from the respective performance in each of the addiction-like criteria ($n = 8$ 0crit, $n = 27$ 1crit, $n = 17$ 2crit, $n = 8$ 3crit). (**b**) Low frequency stimulation (10 Hz 10 min, arrow) induced eCB-LTD of excitatory postsynaptic currents (EPSC) in controls ($n = 8$; $W = 36$, $P = 0.003$) but not in 0crit ($n = 8$; $W = 14$, $P = 0.19$) and 3crit rats ($n = 5$; $W = 1$, $P = 0.5$). The three groups differed accordingly (analysis of variance (ANOVA), Group effect, $F(2,18) = 3.95$, $P < 0.05$, *post hoc*: $P < 0.05$ for 3crit vs control and 0crit vs control). Representative EPSC traces are depicted: (1) baseline, (2) after LTD induction; stimulation artifacts were truncated. Calibration: 25 ms, 100 pA.

Table 1. Cocaine consumption was similar across the different experimental groups

	Last 3 days (infusion per day)	*Lifetime consumption (infusion per day)*
Addiction Resistant rats (mGluR2/3-LTD)	26.1 ± 0.4	25.3 ± 0.86
Addiction Vulnerable rats (mGluR2/3-LTD)	29 ± 0.85	28.5 ± 1.17
Addiction Resistant rats (eCB-LTD–AMPA/NMDA ratio)	30.5 ± 1.15	26.4 ± 1.09
Addiction Vulnerable rats (eCB-LTD–AMPA/NMDA ratio)	30.74 ± 1.6	26.55 ± 1.28
Non-Addict-like (0crit)	27.1 ± 1.34	28.24 ± 1.36
Addict-like (3crit)	31.2 ± 1.85	31.1 ± 1.21

Abbreviations: eCB-LTD, endocannabinoid-mediated long-term synaptic depression; mGluR2/3-LTD; metabotropic glutamate receptor 2/3-mediated LTD; SA, self-administration.
Average daily cocaine intake during the last 3 days of SA and mean daily consumption averaged over lifetime are depicted.

Impairment of mGluR2/3-LTD is a specific marker of cocaine addiction-like behaviors

Metabotropic glutamate receptors (mGluR) are prominent actors of synaptic plasticity throughout the central nervous system and particularly in reward-related areas such as the PFC and the NAC.[34,35] Presynaptic mGluR2/3 are negatively coupled to the cAMP/PKA pathway and their activation triggers a robust LTD.[35] Recently, converging evidence have suggested a role for mGluR2/3 in controlling drug-related behaviors.[15,36]

Bath perfusion with the specific mGluR2/3 agonist LY379268 (100 nM) induced a robust LTD at pPFC excitatory synapses in controls (68.1 ± 4.8% of baseline, $P = 0.03$, Figure 2a). In marked contrast with the eCB-LTD, 0crit rats showed a normal mGluR2/3-LTD (72 ± 4% of baseline, $P < 0.01$, Figure 2a), while it was totally abolished in 3crit animals (97.1 ± 8.5% of baseline, $P = 0.15$, Figure 2a). Thus, the experimental groups differed ($F(2,17) = 6.08$, $P < 0.01$) for the amplitude of the response after LTD induction, with 0crit rats and controls being similar and both differing from 3crit rats ($P < 0.01$ for both). Moreover, the magnitude of the normalized fEPSP after LTD induction correlated with the individual addiction scores ($r = 0.78$, $P < 0.001$, Figure 2b). Such correlation reinforces the idea that adaptations in mGluR2/3-mediated synaptic functions are involved in the behavioral expression of the addiction-like criteria.

There was also a specific reduction of mGluR2/3 protein levels in the dorsomedial PFC of 3crit rats (optical density, controls: 5.91 ± 1.51; 0crit: 8.21 ± 1.97; 3crit: 2.4 ± 0.6; $^*P < 0.05$) (Figure 2c). This finding provides a plausible mechanism for the loss of mGluR2/3-LTD that characterizes Addict-like rats.

The absence of mGluR2/3-LTD is the first synaptic marker in the pPFC specifically associated with the behavioral manifestation of addiction-like behaviors in preclinical models.

Transition to addiction is associated with a postsynaptic plasticity of pPFC excitatory synapses

The previous experiments show that 3crit rats lack two important presynaptic activity-dependent mechanisms to depress glutamate release. We next assessed whether cocaine addiction-like behaviors were also associated with postsynaptically expressed changes in basal synaptic strength. This can be reliably estimated across groups by comparing the ratio of AMPAR-mediated EPSC with NMDAR-mediated EPSC (AMPA/NMDA ratio).[12] In the ventral tegmental area, the NAC and the bed nucleus of stria terminalis, this robust method has been previously used to determinate whether excitatory synapses were potentiated or depressed by cocaine.[37–39]

We found group differences in the AMPA/NMDA ratio ($F(2,17) = 4.03$, $P < 0.05$). While it was similar between controls and 0crit rats (0.69 ± 0.08 and 0.59 ± 0.13, respectively, Figure 3a), the AMPA/

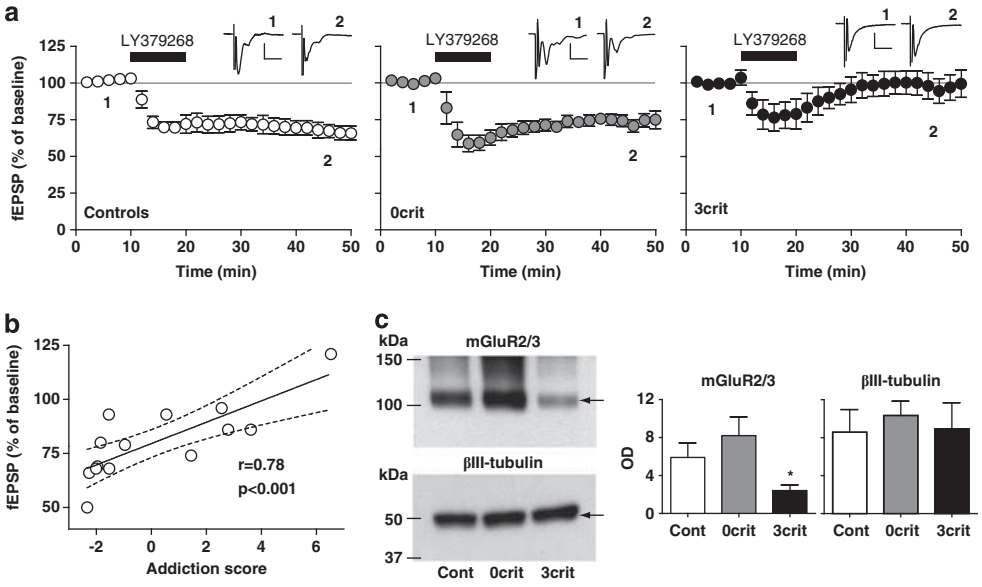

Figure 2. mGluR2/3-dependent long-term synaptic depression (LTD) was selectively impaired in Addict-like (3crit) rats. (**a**) Bath application of the specific mGluR2/3 agonist LY379268 (100 nM, 10 min, black bar) induced LTD of field excitatory postsynaptic potential (fEPSP) in both controls ($N=5$; $W=15$, $P=0.03$) and 0crit ($N=8$; $W=36$, $P<0.01$) rats. In contrast, mGluR2/3-LTD was completely abolished in 3crit rats ($N=7$; $W=14$, $P=0.15$). The three groups differed accordingly (analysis of variance (ANOVA), Group effect, $F(2,17)=6.08$, $P<0.01$, *post hoc*: $P<0.05$ for 3crit vs controls and 3crit vs 0crit). Representative fEPSP traces recorded during baseline (1) and 35 min after LTD induction (2) are depicted. Stimulation artifacts were truncated. Calibration bars: 10 ms, 0.2 mV. (**b**) The magnitude of mGluR2/3-LTD significantly correlated with the individual addiction scores. (**c**) Comparison of the expression of mGluR2/3 proteins. Left: western blot analysis of dorsomedial prefrontal cortex (PFC) extracts for mGluR2/3 (loading control: βIII-tubulin). Right: densitometry quantification (optical density, OD) of the corresponding X-ray films (Control: $N=5$, 0crit: $N=8$, 3crit: $N=5$, $^*P<0.05$).

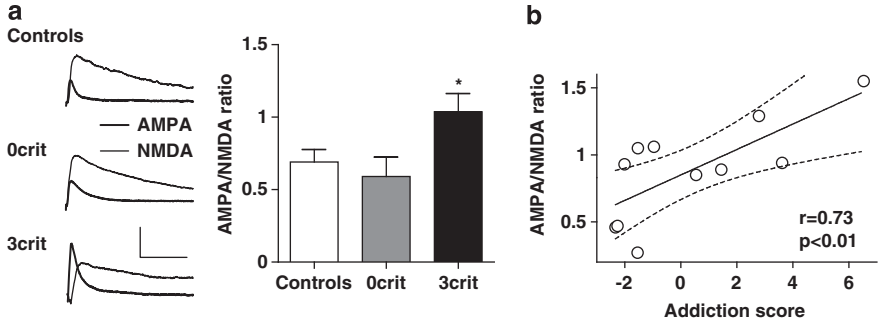

Figure 3. Addict-like (3crit) rats show enhanced AMPA/NMDA ratio at synapses on pyramidal cells of the prelimbic PFC (pPFC). (**a**) Left: representative traces of evoked excitatory postsynaptic currents (EPSC) at +40 mV (calibration bars: 50 ms, 50 pA). Right: summary of AMPA/NMDA ratio obtained from controls ($N=8$), 0crit ($N=6$) and 3crit ($N=6$) rats. The AMPA/NMDA ratio was markedly enhanced in 3crit rats (analysis of variance (ANOVA), Group effect, $F(2,17)=4.03$, $P<0.05$; *post hoc*: $^*P<0.05$ for 3crit vs controls and 3crit vs 0crit). (**b**) AMPA/NMDA ratio was linearly correlated with the addiction score.

NMDA ratio was increased by about 50% in pPFC cells of 3crit rats (1.03 ± 0.12, $P<0.05$, as compared with either controls or 0crit). Moreover, there was a significant correlation between AMPA/NMDA ratio and individual addiction scores ($r=0.73$, $P<0.01$, Figure 3b). This postsynaptic plasticity was observed in the absence of modifications in basal glutamate release, as evidenced by measuring the paired-pulse ratio of evoked EPSC (Supplementary Figure S6). Together, our data show that cocaine addiction-like state is characterized by a substantial adjustment of postsynaptic glutamate receptors that mediate basal synaptic strength in the pPFC.

Addiction-associated synaptic changes in the pPFC are not fully developed during early stages of cocaine SA

We have previously shown that transition to cocaine addiction-like behavior was associated with a persistent loss of NMDAR-dependent LTD (NMDAR-LTD) in the NAC. Thus, 17 days of cocaine SA disrupted NMDAR-LTD in all subjects, independently of the vulnerability to develop addiction. After 2 months of cocaine SA, NMDAR-LTD had recovered in 0crit rats while it was still impaired in 3crit animals.[11]

We analyzed if a similar phenomenon occurred in the pPFC. To this purpose, we used a procedure that allows identifying at an early stage of SA the animals with low (Resistant) or high (Vulnerable) risk to undergo transition to addiction.[11] Animals in these two groups were tested for the expression of synaptic plasticity 24 h after 17 days of cocaine SA. Rats, identified as Addiction Vulnerable and Resistant, self-administered similar cocaine amounts over the 17 training SA sessions before they were killed for *ex-vivo* electrophysiology (Table 1).

As compared with the age-matched controls, the presynaptic and postsynaptic parameters studied were unaltered in both

Resistant and Vulnerable rats. Thus, eCB-LTD and mGluR2/3-LTD were similar between controls, Resistant and Vulnerable rats (Figures 4a and b; $F(2,22) = 0.32$, $P = 0.72$; $F(2,15) = 0.22$, $P = 0.81$; respectively). All groups produced a significant LTD (eCB-LTD: controls: $67.8 \pm 6.9\%$, $P = 0.008$; Resistant: $73.1 \pm 6.5\%$, $P = 0.01$; Vulnerable: $75 \pm 6.6\%$, $P = 0.01$; mGluR2/3-LTD: controls: $45.2 \pm 5.2\%$, $P = 0.01$; Resistant: $52.3 \pm 9.5\%$, $P = 0.01$; Vulnerable: $50.3 \pm 8.1\%$, $P = 0.01$). Similarly, the three groups did not differ for the AMPA/NMDA ratio ($F(2,28) = 2.35$, $P = 0.11$) which was comparable in controls (0.65 ± 0.06), Resistant (0.68 ± 0.05) and Vulnerable (0.84 ± 0.08) animals (Figure 5). Nonetheless, there was a strong correlation between the AMPA/NMDA ratio and the two main behavioral criteria that predict vulnerability to undergo transition to addiction ($r = 0.78$, $P = 0.0002$; $r = 0.77$, $P = 0.002$; Figure 5). The increase in AMPA/NMDA ratio observed in the 3crit rats may not yet be fully established in Vulnerable rats after 17 cocaine SA sessions, but may develop progressively and concomitant with the slow development of addiction-like behaviors. These data reveal that overall addiction-associated impairments occur later in the pPFC than in the NAC.

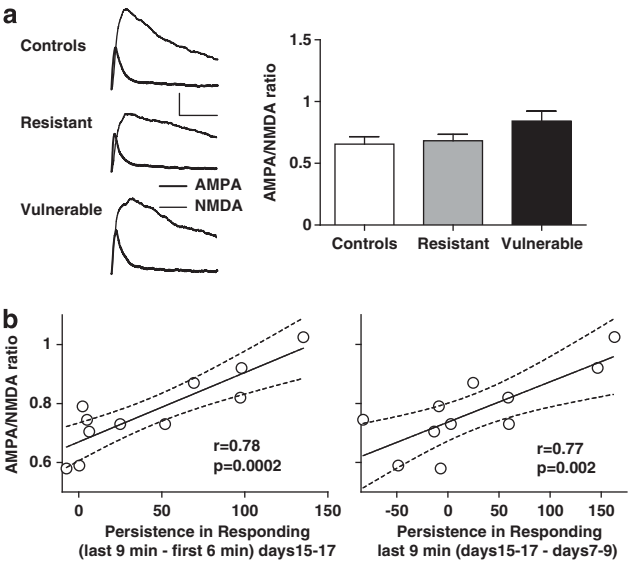

Figure 5. AMPA/NMDA ratio in the prelimbic PFC (pPFC) after 17 days of cocaine self-administration (SA). (**a**) The AMPAR/NMDAR ratio at pyramidal pPFC synapses was not significantly modified after 17 days of cocaine SA. Left: representative traces of evoked excitatory postsynaptic currents (EPSC) at $+40$ mV (calibration: 50 ms, 50 pA). Right: summary of AMPAR/NMDAR ratio obtained from controls ($N = 10$), Resistant ($N = 10$) and Vulnerable ($N = 11$) animals. The three groups showed a comparable ratio (ANOVA, group effect, $F(2,28) = 2.35$, $P = 0.11$). (**b**) There was a strong and significant correlation between the AMPA/NMDA ratio and the performance on the two main behavioral criteria that predict the vulnerability to undergo transition to addiction. Left: difference between the active responses during the last 9 min and the first 6 min averaged over sessions 15–17. Right: difference between the average active responses during the last 9 min of sessions 15–17 and sessions 1–5.

DISCUSSION

Identifying the drug-induced neurobiological change(s) specifically associated with the behavioral manifestations of addiction has proven to be extremely challenging[7] but the recent development of adequate animal models[1,8,11,40] will allow rapid progress. By differentiating between individuals (the vast majority) maintaining control of their drug-related behaviors from those few that lose control over drug consumption, we show here that transition to addiction is specifically associated with an impairment in deep layers pPFC excitatory synapses. Specifically, in Addict-like rats mGluR2/3-LTD was abolished and there was a concomitant postsynaptic plasticity of basal synaptic strength. In contrast, despite a similar amount of self-administered cocaine,

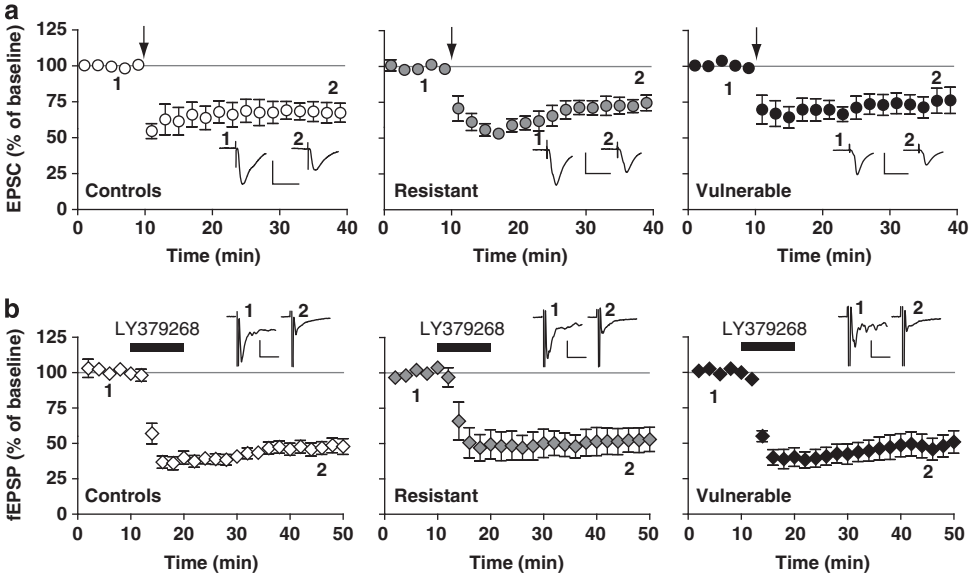

Figure 4. Presynaptically expressed plasticity is unchanged during early stages of cocaine self-administration (SA). (**a**) Endocannabinoid-mediated long-term synaptic depression (eCB-LTD) triggered with a stimulation train at a natural occurring frequency (10 Hz for 10 min, arrow) was normal in controls ($N = 10$; $W = 53$, $P = 0.008$), Resistant ($N = 7$; $W = 28$, $P = 0.01$) and Vulnerable ($N = 8$; $W = 34$, $P = 0.01$) rats. The three groups showed a comparable LTD induction (analysis of variance (ANOVA), Group effect, $F(2,22) = 0.32$, $P = 0.72$). (**b**) The specific mGluR2/3 agonist LY379268 (100 nM, 10 min, black bar) induced LTD of field excitatory postsynaptic potential (fEPSP) in controls ($N = 6$; $W = 21$, $P = 0.01$), Resistant ($N = 6$; $W = 21$, $P = 0.01$) and Vulnerable ($N = 6$; $W = 21$, $P = 0.01$) rats. The three groups showed a comparable LTD induction (analysis of variance (ANOVA), Group effect, $F(2,15) = 0.21$, $P = 0.81$). In (**a**) and (**b**), representative excitatory postsynaptic currents (EPSC)/fEPSP traces are depicted: (1) baseline, (2) after LTD induction; stimulation artifacts were truncated. Calibration: 25 ms, 100 pA; 10 ms, 0.2 mV.

Non-Addict-like rats did not differ from drug naive animals for these two parameters.

The specific impairment of mGluR2/3-LTD in animals developing an addiction-like behavior provides new insights into the role of mGluR2/3 during the addiction process. Previous observations have shown that mGluR2/3 participate to cocaine-related behaviors and that pharmacological stimulation of mGluR2/3 reduces cocaine SA and drug-seeking behavior.[41–43] Conversely, inhibition of mGluR2 signaling by genetic deletion of mGluR2 enhances cocaine conditioned place preference.[44] Our study further suggests that a downregulation of mGluR2/3 protein and function triggered by prolonged cocaine experience may confer vulnerability to develop addiction-like behaviors. This hypothesis supports the idea that targeting this receptor pathway may open new therapeutic strategies of addiction.[36,45–47]

The mechanisms of mGluR2/3 dysfunction in Addict-like rats remain to be fully identified. In addition to changes in mGluR2/3 content, deficiencies in mGluR2/3 intracellular signaling may be involved. In fact, previous studies have shown that protracted withdrawal from cocaine experience modifies Giα-coupled receptors signaling, including mGluR2/3, through an elevation of the Activator of G protein Signaling 3.[48,49]

It was previously shown that repeated non-contingent cocaine administration disrupted mGluR2/3-LTD in rats PFC.[34] In our experimental conditions, mGluR2/3-LTD was found normal in all animals following 17 days of cocaine SA, and in the majority of them after months (that is, in Non-Addict-like rats). These data provide further evidence that non-contingent administration of drugs and SA have different consequences.[50–53]

By measuring the AMPA/NMDA ratio, we uncovered a specific postsynaptic plasticity in the pPFC of Addict-like animals. Although it is difficult to draw absolute conclusions on changes in AMPAR-mediated and NMDAR-mediated currents solely based on AMPA/NMDA ratio, these results may imply a potentiation of AMPAR-mediated synaptic transmission. Mechanistically, this postsynaptic plasticity could be a direct result of the impaired presynaptic LTD. The lack of eCB-LTD and mGluR2/3-LTD in the pPFC may prevent those synapses to dynamically downregulate glutamate release under situations of intense neuronal activity. Thus, the absence of two major LTD-inducing paradigms in Addicts may favor the induction of a postsynaptic, presumably LTP-like phenomenon. In addition, a decreased GABAergic inhibition of pPFC pyramidal cells may also contribute to the expression of a putative LTP, as previously shown upon repeated cocaine exposure.[54]

An enhancement of PFC neuronal activity has been observed in rats during cocaine SA.[16] More importantly, human imaging studies have shown that the PFC of individuals addicted to drugs of abuse was prominently activated when they were exposed to cues previously associated with drug use and correlated with self-reported drug craving.[17] The postsynaptic plasticity of basal synaptic transmission and the impaired negative regulation of glutamate release observed here in Addict-like rats may serve as a cellular mechanism by which PFC neurons recruited in a context-specific manner (that is, drug-related) can trigger drug-seeking behaviors in an uncontrolled manner. Our data, however, do not exclude a contribution of other adaptations, such as changes in intrinsic excitability of PFC neurons.[55]

Different subregions of the medial PFC are considered to have opposite roles in the control of drug-related behaviors. Numerous studies suggest that pPFC may promote, whereas infralimbic PFC may inhibit cocaine seeking.[19–21] In this context, it is tempting to speculate that divergent synaptic adaptations may occur in pPFC and infralimbic PFC of Addict-like animals, which together may mediate the loss of control over drug-related behaviors.

eCB-dependent synaptic plasticity was similarly impaired in the pPFC of Addict- and Non-Addict-like animals after prolonged cocaine SA. In the pPFC as in other brain regions, eCB-LTD results from the stimulation of postsynaptic group I mGluR and the consequent synthesis and release of eCB that inhibit neurotransmitter release via CB1R.[56] Our data are in agreement with previous reports showing that cocaine interferes with group I mGluR-mediated plasticity and functions.[33,46,57,58] Hence, alterations of group I mGluR-mediated synaptic plasticity in the mesocorticolimbic system seem to be a widespread consequence of chronic cocaine intake, probably important in mediating the reinforcing effects of drugs,[31,32] but not specifically associated with transition to addiction.

Taken together, the present results and our previous report[11] support the idea that abnormal synaptic plasticity could participate to transition to addiction. The large impairment in synaptic plasticity observed in Addict-like rats could explain their loss of control on drug intake. Indeed, activity-dependent synaptic plasticity is thought to have an important role in the refinement of neuronal circuits necessary to adapt and change behavior in response to environmental contingencies. In Addict-like rats, the lack of LTD may underlie a persistent inability to down scale synapses previously potentiated by drug experience.[6,50,59,60] Thus, the lack of LTD and the associated change of basal synaptic strength could be the signature of addicted synaptic circuits resistant to modulation by environmental contingencies. These synaptic modifications could be responsible for behavioral inflexibility that would culminate in the loss of control over drug seeking and taking.

These results in the pPFC also extend previous findings in the NAC. Together, they allow proposing that transition to addiction is associated with two types of plastic adjustments.[11] The first, termed *anaplasticity* refers to the inability of Addict-like rats to adapt to drug-induced alterations that initially occur in all users and are exclusively reversed in rats maintaining a controlled drug intake (that is, NMDAR-LTD in the NAC). The second, illustrated by our present results, refers to drug responses occurring exclusively in Addict-like rats (that is, the loss of mGluR2/3-LTD and postsynaptic plasticity in the pPFC).

It was suggested that cocaine-induced changes in synaptic plasticity may be sequential and hierarchically progress over time from the ventral tegmental area to the NAC and afterwards to the PFC.[61] Our data are in agreement with the sequential expression of synaptic adaptations, showing that modifications in the NAC appear earlier than in the pPFC. NMDAR-LTD is impaired at early stages of cocaine SA in the NAC of all individuals while addiction-associated adaptations in the pPFC are not fully developed in Vulnerable rats. Later on, when addiction-like behaviors appear, Addict-like rats maintain an impaired NMDAR-LTD and develop a specific impairment in the pPFC. If and how cocaine-induced *anaplasticity* in the NAC and addiction-specific changes in the pPFC are molecularly independent or hierarchically related phenomena remains to be elucidated.

In conclusion, addiction seems to be the result of a dynamic interplay between the inability to recruit active compensatory mechanisms (*anaplasticity*) and the emergence of specific neuronal impairments that led to the loss of synaptic plasticity. Clarifying, the nature of the interactions between these phenomena and their specific roles during the transition to addiction will help to identify the best molecular targets for efficient therapies of addiction.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Author contributions: FK and ML performed the electrophysiology experiments, conducted the data analyses and contributed to the design of the experiments. VD-G designed and supervised the behavioral experiments, and conducted the data analyses. J-MR designed and conducted the immunoblotting analyses. NB, EB, J-FF and PR conducted the behavioral experiments. P-VP and OJM supervised the entire project. FK, VD-G, P-VP and OJM wrote the manuscript.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)