Pharmacological Reports 71 (2019) 870–878

Contents lists available at ScienceDirect

Pharmacological Reports

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

Original article

Extinction training following cocaine or MDMA self-administration produces discrete changes in D_2-like and $mGlu_5$ receptor density in the rat brain

Małgorzata Frankowska[a,*], Joanna Miszkiel[a], Lucyna Pomierny-Chamioło[b], Bartosz Pomierny[b], Andrea Celeste Borelli[c], Agata Suder[a], Małgorzata Filip[a]

[a] Maj Institute of Pharmacology, Polish Academy of Sciences, Department of Drug Addiction Pharmacology, Kraków, Poland
[b] Department of Toxicology, Faculty of Pharmacy, Jagiellonian University Collegium Medicum, Kraków, Poland
[c] Department of of Medical Sciences, University of Ferrara, Ferrara, Italy

ARTICLE INFO

Article history:
Received 4 February 2019
Received in revised form 11 April 2019
Accepted 6 May 2019
Available online 7 May 2019

Keywords:
Cocaine self-administration
MDMA self-administration
Extinction training
Reinstatement of seeking-behavior
Dopamine D_2-like receptor
Glutamate $mGlu_5$ receptor

ABSTRACT

Background: Several studies strongly support the role of the dopamine D_2-like and glutamate $mGlu_5$ receptors in psychostimulant reward and relapse.
Methods: The present study employed cocaine or MDMA self-administration with yoked-triad procedure in rats to explore whether extinction training affects the drug-seeking behavior and the D_2-like and $mGlu_5$ receptor B_{max} and K_d values in several regions of the animal brain.
Results: Both cocaine and MDMA rats developed maintenance of self-administration, but MDMA evoked lower response rates and speed of self-administration acquisition. During reinstatement tests, cocaine or MDMA seeking behavior was produced by either exposure to the drug-associated cues or drug-priming injections. The extinction training after cocaine self-administration did not alter significantly D_2-like receptor expression the in the limbic and subcortical brain areas, while MDMA yoked rats showed a decrease of the D_2-like binding density in the nucleus accumbens and increase in the hippocampus and a rise of affinity in the striatum and hippocampus. Interestingly, in the prefrontal cortex a reduction in the $mGlu_5$ receptor density in cocaine- or MDMA-abstinent rats was demonstrated, with significant effects being observed after previous MDMA exposure. Moreover, rats self-administered cocaine showed a rise in the density of $mGlu_5$ receptor for the nucleus accumbens.
Conclusion: This study first time shows that abstinence followed extinction training after cocaine or MDMA self- or passive-injections changes the D_2-like and $mGlu_5$ density and affinity. The observed changes in the expression of both receptors are brain-region specific and related to either pharmacological and/or motivational features of cocaine or MDMA.

Introduction

Substance use disorder (SUD) is a chronic disease characterized by compulsive, uncontrollable drug seeking and drug taking which produces persistent neuroadaptions in the brain that promote vulnerability to relapse to drug use following abstinence. Despite many years of research, effective medications and behavioral interventions to prevent relapse and facilitate longer periods of abstinence are still looked for. Results from European studies estimated the prevalence rates between 40 and 75% for illicit drugs in the first year of hospitalization [1,2]. Chronic drug use prominently disrupts homeostasis in the brain reward circuitry enhancing motivated seeking behavior for natural or drug rewards. The reward circuitry includes the ventral tegmental area, nucleus accumbens, prefrontal cortex, dorsal striatum, amygdala and hippocampus [3–5]. It is well-documented that dopamine is a key neurotransmitter that mediates reward property and drug-seeking behavior [3–6]. Nevertheless, extensive studies carried out in the last three decades demonstrated the role of the excitatory neurotransmitter glutamate in several aspects of drug addiction, including drug reward and drug seeking [7–9].

The primary mode of action of the most widespread recreational drugs of abuse, such as cocaine and 3,4-methylene-dioxymethamphetamine (MDMA) depends on an increase

* Corresponding author.
E-mail address: frankow@if-pan.krakow.pl (M. Frankowska).

http://dx.doi.org/10.1016/j.pharep.2019.05.001
1734-1140/

extracellular level of dopamine by blocking the transporter involved in its reuptake (cocaine) as well as an enhancement of its release (MDMA); both drugs also increase the extracellular level of serotonin (5-HT) and noradrenaline [10,11]. MDMA increases the release of monoamine neurotransmitters also by direct action on presynaptic vesicles [12–14]. Interestingly, as compared to cocaine, MDMA in primate brain predominantly affects 5-HT neurons and induces damage of 5-HT axons and nerve fibers and intracytoplasmic inclusions [15–17]. Both psychostimulants also increase extracellular levels of glutamate which plays an important role in the long-term molecular and behavioral changes underlying drug addiction [7].

In the present paper, we investigated how brain dopamine D_2-like and glutamatergic $mGlu_5$ receptor density and affinity in rats change during cocaine and MDMA self-administration and drug-free period with extinction training. These receptor sites were chosen since: (i) imaging human studies have demonstrated reduced levels of brain postsynaptic dopamine $D_{2/3}$ receptor among drug abusers [18], (ii) in preclinical research drugs of abuse reduced availability of dopamine D_2 receptors in the rodent basal ganglia while animals with lower dopamine D_2 receptor density were especially responsive to cocaine's reinforcing effects [19], (iii) extensive preclinical pharmacological studies have shown that glutamate $mGlu_5$ receptors are a target for the rewarding properties of drugs of abuse [see ref. 20], (iv) mice lacking glutamate $mGlu_5$ failed to intravenously self-administer cocaine and to show any hyperactivity after acute administration of the drug [21], and (v) in the striatum, the brain area necessary for habit-formation during drug addiction, the occurrence of oligomers of $mGlu_5$ and D_2 with adenosine A_{2A} receptors has been detected [22]. Receptor density (a total concentration of receptors in a sample of tissue; B_{max}) and sometimes affinity (expressed as constants for association and dissociation of the ligand to and from the receptors; K_d) for agonists and antagonists present a dynamic processes and play important role in receptor–drug interactions. Baseline receptor density and affinity vary from tissue to tissue; they can be influenced by extracellular (e.g., drugs of abuse) as well as by intracellular (e.g., neurotransmitters) factors leading to their changes (up- or downregulation).

Material and methods

Animals

Experimentally naive male Wistar rats (225–250 g; N = 94) delivered by a licensed breeder (Charles River, Munich, Germany) were housed individually in standard plastic rodent cages in a room maintained at 22 ± 2 °C and 40–50% humidity under a 12-h light-dark cycle (lights on at 6.00 a.m.). Animals had free access to food (Labofeed pellets) and water during a 7-day habituation period. Following habituation, the rats were maintained on limited access to water during the initial training sessions, as described previously [23]. All experiments were conducted during the light phase of the light-dark cycle (between 8.00 a.m. and 3.00 p.m.), and experiments were carried out in accordance with the European Directive 2010/63/EU and were approved by the Ethical Committee at the Maj Institute of Pharmacology, Polish Academy of Sciences, Krakow.

Behavioral experiments

Drugs

Cocaine HCl (National Institute on Drug Abuse, RTI International, USA) and 3,4-methylenedioxymethamphetamine HCl (MDMA, THC Pharm GmbH, Frankfurt/Main, Germany) were dissolved in sterile 0.9% NaCl and given *iv* (0.1 ml/infusion) or *ip* (1 ml/kg).

Lever-press training and surgery

After a week of habituation to the animal facility, all animals were water-deprived for 18 h and subsequently trained to press the lever for 2 h daily for water reinforcement on a fixed ratio (FR) 1 schedule of reinforcement. For cocaine self-administration on the fourth day of lever press training, the number of responses required to produce reinforcement increased from 1 to 3, and after other 2 days from 3 to 5. During this phase of training, the amount of water each animal received was restricted to that given during daily training sessions, and animals were allowed a 10-min free access to water in home cages after training sessions. Two days after the lever-press training and after giving the animals free access to water, the rats were anesthetized with ketamine HCl (75 mg/kg; Bioketan; Biowet, Pulawy, Poland) and xylazine (5 mg/kg; Sedazin; Biowet, Pulawy, Poland), and chronically implanted with a silastic catheter in the external jugular vein, as described previously [23]. After catheter implantation and recovery rats were kept individually in standard rat home cages with free access to water and food. Catheters were flushed every day with 0.1 ml of saline solution containing heparin (70 U/ml) and/or 0.1 ml of cephazolin solution (10 mg/ml; Biochemie GmbH, Kundl, Austria). Animals in which problems with the catheters were observed during the recovery period were removed from experiments (N = 2).

Apparatus

Cocaine or MDMA self-administration experiments were conducted in standard operant chambers (Med-Associates, St. Albans, USA), as descried previously [23].

Experimental procedures

Self-administration. Rats were allowed 8–10 days to recover from surgical procedures before the start of the experiments. Later, all animals deprived of water for 18 h were trained in one 2-h session to press the lever for water reinforcement on an FR5 for cocaine or FR1 for MDMA schedules. Then, animals began lever pressing for cocaine or MDMA reinforcement and from that time on they were given water *ad libitum* throughout the remainder of the experiment. Rats were given access to cocaine or MDMA during 2-h daily sessions performed 6 days/week (maintenance) (Figs. 1A and B). Concurrent with infusion for cocaine or MDMA presented for 5 s, the conditioned stimulus, a tone (2000 Hz; 15 dB) and illumination of the stimulus light directly above the active lever, were presented. Following each drug injection there was a 20 s time-out period during which responding was recorded but had no programmed consequences. Response on the inactive lever never resulted in cocaine or MDMA delivery.

For cocaine self-administration experiments (N = 22) each completion of FR5 schedule of reinforcement resulted in an infusion of cocaine a dose 0.5 mg/kg. Each training trial lasted for 2 h and the animal during maintenance had self-administered at least 25 infusions of cocaine. An arbitrary acquisition criterion required that active lever presses vary by 10% or less over 3 consecutive days during maintenance. The experiment was performed as described previously [23].

Initial training for MDMA self-administration (N = 30) consisted of daily sessions during which each active lever press was reinforced with an MDMA infusion at a dose 1 mg/kg according to FR1 schedule of reinforcement [24]. Responses maintained by MDMA (1 mg/kg) are generally low [24,25] and following acquisition (at least 2 consecutive days of at least 10 active lever responses and a preference for the active lever), the dose of MDMA was reduced to 0.5 mg/kg/infusion [24]. After 10 days of MDMA self-administration, the response requirements were increased to FR3 till the end of the experiment. An arbitrary acquisition

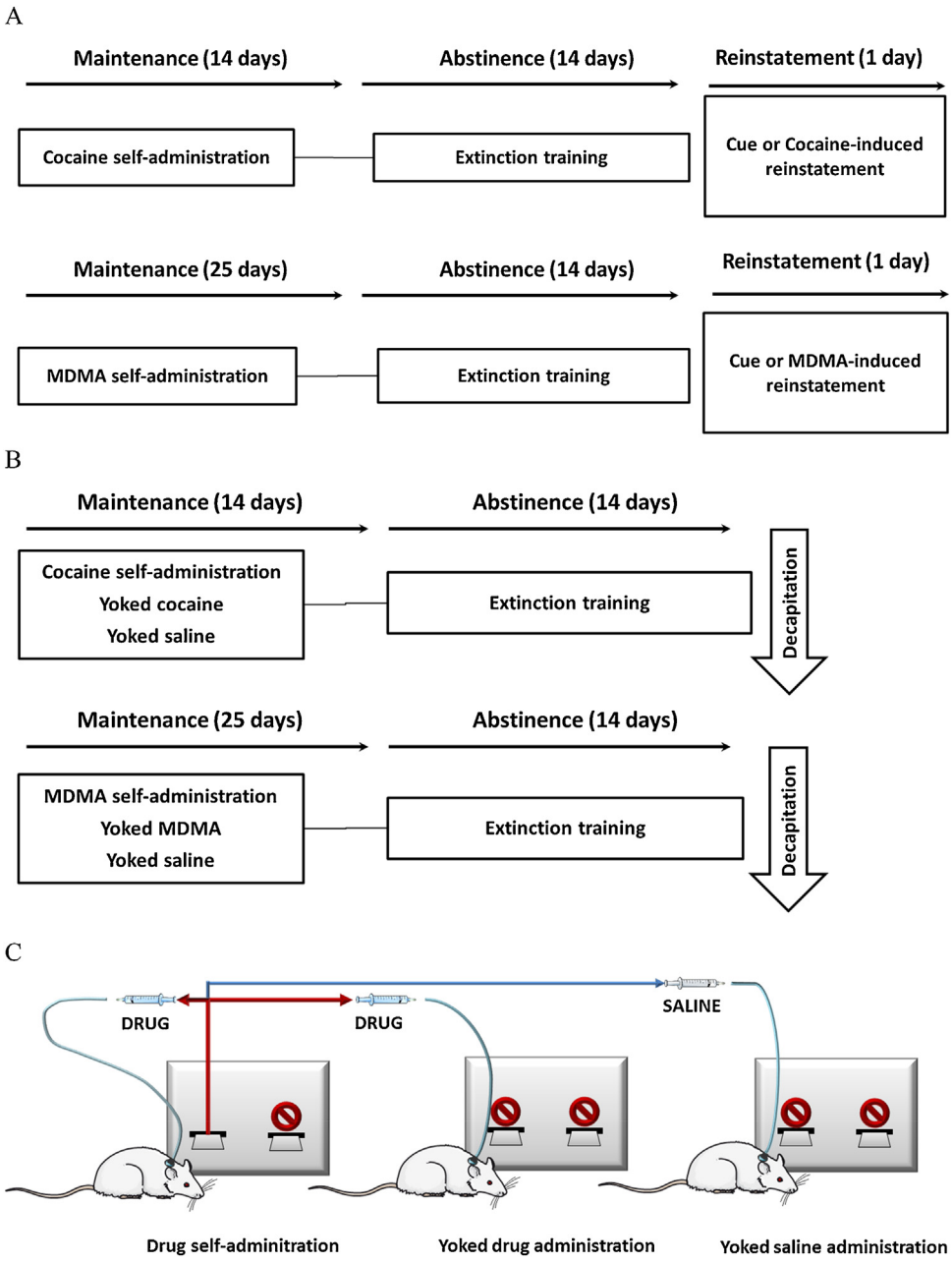

Fig. 1. Experimental design for behavioral (A) and molecular (B) analyses and scheme for yoked procedure (C).

criterion required that active lever presses varied by 15% or less over 3 consecutive days during maintenance, and not less than 10 infusions of MDMA self-administered during session. Between training sessions animals were keep individually in standard home cages, and were handled daily

Extinction training and reinstatement of seeking-behavior. After cocaine or MDMA self-administration (once the rats met the maintenance criterion), animals underwent a 14-day extinction trial in experimental cage. During extinction, the animals experienced 2-h daily training sessions; however, active lever presses resulted neither in the delivery of cocaine or MDMA (saline was substituted for drug) nor the presentation of the conditioned stimulus. Following 14-daily extinction training, the number of active lever presses was below 15% for cocaine or 25% for MDMA compared to the number of active lever presses reached during maintenance. Between training sessions animals were kept individually in standard home cages and were handled minimum once per day. On the 14th day of extinction, a part of the above cohort (N = 10 rats for cocaine and N = 9 rats for MDMA) was tested for the response reinstatement induced by either a conditioned cue (the tone + light) previously paired with cocaine or MDMA self-administration or a non-contingent presentation of cocaine (10 mg/kg, *ip*) or MDMA (2.5–10 mg/kg, *ip*). During the reinstatement tests (2-h sessions), active lever presses resulted in an intravenous injection of saline only. A maximum of four test sessions was performed on each rat, separated by at least two baseline days of extinction training session. Another part of cohort was sacrificed immediately following the last (14th) extinction session (2 groups of rats with N = 10).

Yoked self-administration procedure. For biochemical experiments yoked self-administration procedure was used. Rats were tested

simultaneously in groups of three with two rats serving as yoked controls that received an injection of cocaine or MDMA or saline which was not contingent on responding, each time a response-contingent injection of cocaine or MDMA was self-administered by the paired rat (see Figs. 1B and C). Unlike self-administering rats, lever pressing by the yoked rats was recorded but had no programmed consequence. Yoked saline and yoked cocaine or yoked MDMA animals were sacrificed after 14 days of extinction training, at the same time as corresponding groups of rats self-administering cocaine or MDMA (2 yoked saline groups N = 10; yoked cocaine group N = 10; yoked MDMA group N = 10).

Biochemical experiments

Saturation binding assay

Dissection. Animals, underwent self-administration (at least 14 days for cocaine and 25 days for MDMA) and 14-day extinction trials, were sacrificed immediately following the last session of extinction. Each animal was decapitated and its brain was quickly removed and chilled in ice-cold saline. The prefrontal cortex, including the infralimbic, prelimbic, cingular cortices (Bregma: 5.2−2.7 mm), the nucleus accumbens, with the shell and core parts (Bregma: 2.2−1.0 mm), the striatum - dorsal part (Bregma: 2.2−1.0 mm) and the hippocampus (Bregma:-1.4–6.7 mm) were dissected according to the Paxinos and Watson's Rat Brain Atlas [26]. Samples were immediately frozen on dry ice and stored at −80 °C.

Radioligand binding experiments for D_2 receptors. Membrane preparation and the D_2-like receptor antagonist [^{3}H]-raclopride ([^{3}*H*]3,5-dichloro-*N*-[[(2S)-1-ethyl-2-pyrrolidinyl]methyl]-2-hydroxy-6-methoxybenzamide; 82.8 Ci/mmol, Perkin Elmer Life Sciences, USA) binding assay were performed as described previously [23].

Radioligand binding experiments for $mGlu_5$ receptors. Membrane preparation and the $mGlu_5$ receptor antagonist [^{3}H]MPEP ([^{3}H]-2-methyl-6-(phenylethynyl)pyridine; 60 Ci/mmol, American Radiolabeled Chemicals, Inc., St. Louis, MO, USA) binding assay were performed as described previously [23,27], with some modifications.

Statistical analysis

Data from the behavioral experiment of acquisition/maintenance of self-administration and extinction training were analyzed using a two-way analysis of variance (ANOVA) with repeated measures to analyze active and inactive lever responses across the transition from one phase to the next conducted for subsequent sessions as factors. Data from reinstatement of seeking behavior tests were analyzed using a two-way analysis of variance (ANOVA) with active and inactive lever responses as factor. Animals which did not complete the self-administration acquisition (N = 2 rats for cocaine and N = 8 rats for MDMA), extinction (N = 3 rats for MDMA) criteria or did not maintain extinction criteria between tests were excluded from tests and the data analysis. Data from the biochemical experiments were analyzed using GraphPad PRISM 5.0 (GraphPad software, San Diego, USA) using a nonlinear regression one-site binding hyperbola. Two parameter values, B_{max} and K_d, were calculated. For statistical evaluation of the biochemical data an one-way ANOVA for each individual group was used. Group differences after significant ANOVAs were analyzed by the *post hoc* Newman-Keuls test. The criterion for a statistically significant difference was set at $p < 0.05$.

Results

Behavioral analysis

Cocaine self-administration, extinction training and reinstatement of drug-seeking

After 14 daily cocaine self-administration sessions, animals showed stable lever-pressing rates during the last 3 self-administration days with less than a 10% difference in their daily intake of cocaine. The mean number of cocaine infusions per day during the last 3 self-administration days varied from 30 to 35, while during 14 experimental sessions, animals received from 159 to 162 mg/kg of cocaine.

Fig. 2 shows the behavioral responses of rats that underwent cocaine self-administration and extinction training. A two-way ANOVA for repeated measures showed a significant effect of the lever ($F(118) = 113.60$, $p < 0.001$), session ($F(23,414)=8.08$, $p < 0.001$) and lever × session interaction ($F(23,414)=12.08$, $p < 0.001$). The *post hoc* analyses revealed a greater frequency of presses on the active lever than on the inactive lever from the 1st cocaine self-administration session until the 1st extinction day ($p < 0.01$). During extinction training when saline was substituted

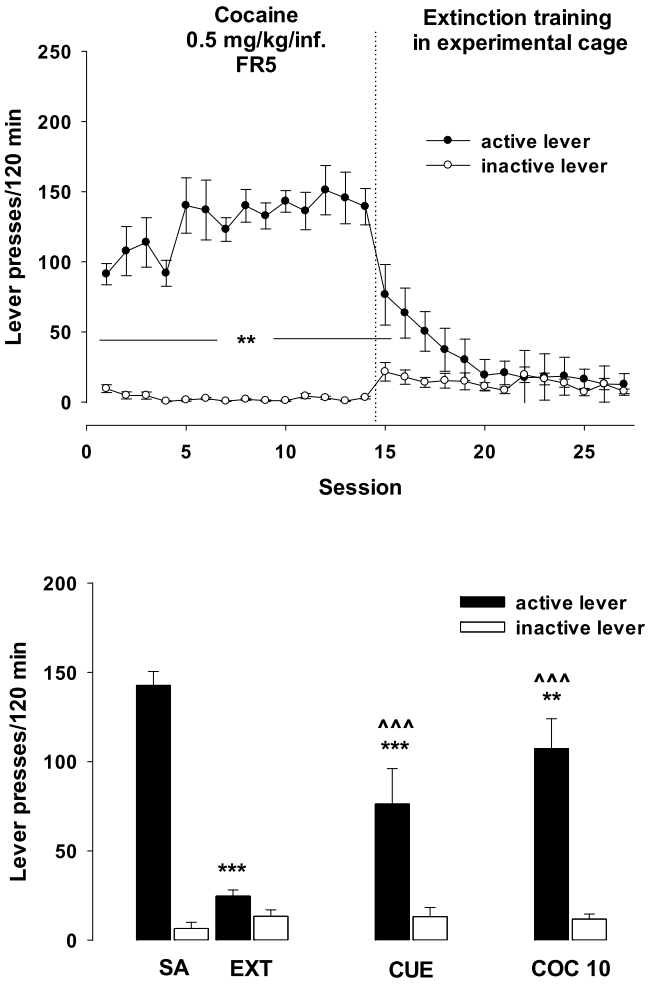

Fig. 2. Representative graph of behavioral responses during acquisition, maintenance of cocaine self-administration, extinction training (*top panel*) and during reinstatement of cocaine-seeking behavior in rats (*bottom panel*). SA – last session of cocaine self-administration; EXT – last session of extinction training; CUE - cue-induced reinstatement; COC - drug-induced reinstatement triggered by administration of cocaine 10 mg/kg (*ip*). All data depict the mean (±SEM) numbers of active and inactive lever presses. N = 8–10 rats/group. $^{**}p < 0.01$; $^{***}p<0.001$ *versus* active lever during SA; $^{\wedge\wedge\wedge}p < 0.001$ *versus* active lever presses during EXT.

for cocaine, a progressive drop in active lever responses was observed. The total number of active lever presses during the last day of extinction was reduced by 85% ($p < 0.001$) compared with the last cocaine self-administration session (Fig. 2 *bottom panel*).

A two-way ANOVA showed a change in lever presses during the reinstatement of drug seeking in rats that previously self-administering cocaine; a significant effect of the cue ($F(242) = 36.44$, $p < 0.001$), lever ($F(142) = 161.79$, $p < 0.001$) and lever × cue interaction ($F(242) = 44.30$, $p < 0.001$) was found. The *post hoc* analyses revealed that the presentation of the cocaine-associated conditioned cue resulted in an increase ($p < 0.001$) in the number of active lever presses compared to the last day of extinction training, but it did not reach the level of responses observed in the last cocaine self-administration session (Fig. 2 bottom panel).

Similar increases were observed for cocaine-induced reinstatement as a two-way ANOVA for active and inactive lever presses revealed a significant effect for treatment × lever interaction ($F(242) = 27.03$, $p < 0.001$), as well as for treatment ($F(242) = 22.86$, $p < 0.001$) and lever ($F(142) = 131.87$, $p < 0.001$). The *post hoc* analyses revealed that cocaine (10 mg/kg, *ip*) priming led to significant enhancement ($p < 0.001$) of active lever responses compared with the last extinction training session (Fig. 2 bottom panel).

MDMA self-administration and extinction training procedures

After 25 daily MDMA self-administration sessions, animals showed stable lever-pressing rates during the last 3 self-administration days with less than a 15% difference in their daily intake of MDMA (Fig. 3). The mean number of MDMA infusions per day during the last self-administration days varied from 13 to 14, while during 25 experimental sessions, animals received from 168 to 185 mg/kg of MDMA.

A two-way ANOVA for repeated measures showed a significant effect of the lever ($F(112) = 33.02$, $p < 0.001$), session ($F(38,456)=6.81$, $p < 0.001$) and lever × session interaction ($F(38,456)=2.44$, $p < 0.001$), while *post hoc* analyses revealed more frequent presses on the active lever than on the inactive lever from the 11th till the last day of MDMA self-administration ($p < 0.05$). After 25 days of MDMA self-administration, extinction training was introduced to the animals. When saline was substituted for MDMA, a progressive drop in lever responses was seen over 14 extinction sessions (Fig. 3 top panel). The total number of active lever presses emitted during the last day of extinction was reduced by 75% ($p < 0.001$).

For the cue-induced reinstatement, a two-way ANOVA analysis revealed a significant effect for the drug-associated conditional stimulus ($F(242) = 8.95$, $p < 0.001$), lever ($F(142) = 55.26$, $p < 0.001$) and lever × cue interaction ($F(242) = 13.38$, $p < 0.001$). The *post hoc* analyses revealed that presentation of the MDMA-associated conditioned cue resulted in an increase ($p < 0.001$) in the number of active lever presses compared to the last day of extinction training, but such increase was lower as compared to the last MDMA self-administration session (Fig. 3 bottom panel).

Pretreatment with MDMA (2.5–10 mg/kg *ip*) resulted in an increase in responses to the active lever during reinstatement tests. The two-way ANOVA for active and inactive lever presses revealed a significant effect for lever in rats with a history of MDMA self-administration ($F(164) = 44.81$, $p < 0.001$) as well as effect for treatment ($F(464) = 5.17$, $p < 0.001$) and lever × treatment interaction ($F(464) = 5.58$, $p < 0.001$). The *post hoc* analyses revealed that injection of MDMA at doses 7.5 and 10, but not at 2.5 mg/kg, resulted in an increase ($p < 0.001$) in responses to the active lever compared to the last extinction training session (Fig. 3 bottom panel), and the value reached the level of responses observed during the last MDMA self-administration session.

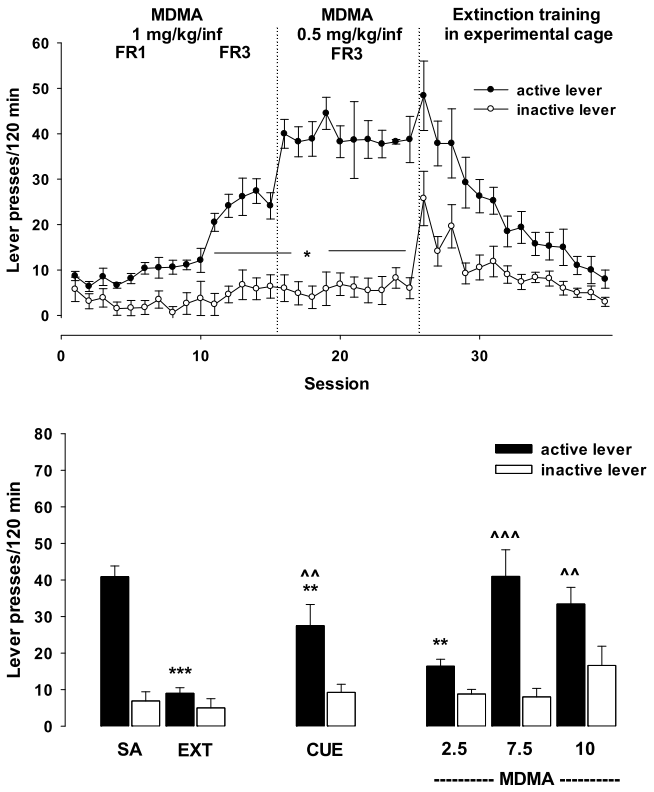

Fig. 3. Representative graph of behavioral responses during acquisition, maintenance of MDMA self-administration, extinction training (*top panel*) and during reinstatement of MDMA-seeking behavior in rats (*bottom panel*). SA – last session of MDMA self-administration; EXT – last session of extinction training; CUE - cue-induced reinstatement; MDMA - drug-induced reinstatement triggered by administration of MDMA 2.5–10 mg/kg (*ip*). All data depict the mean (±SEM) numbers of active and inactive lever presses. N = 7–9 rats/group. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p<0.001$ *versus* active lever during SA; $^{\wedge\wedge}p < 0.01$; $^{\wedge\wedge\wedge}p < 0.001$ *versus* active lever presses during EXT.

Yoked procedure

In the yoked cocaine and yoked saline groups, the difference between pressing the active *versus* the inactive lever failed to reach significance (data not shown). The yoked cocaine animals passively received exactly the same amount of cocaine (from 159 to 162 mg/kg) at the same time as the rats that had learned to actively inject cocaine.

Similar, in the yoked MDMA and yoked saline groups, the difference between pressing the active *versus* the inactive lever failed to reach significance (data not shown). The yoked MDMA animals received exactly the same amount of MDMA (168–185 mg/kg) at the same time as the rats that had learned to actively inject MDMA.

Biochemical analysis

The dopamine D_2-like and glutamate $mGlu_5$ receptor radioligand antagonist binding experiments with D_2/D_3 receptor antagonist ([^{3}H]raclopride) and the radiolabeled $mGlu_5$ receptor antagonist ([^{3}H]MPEP), respectively, in rat brain structures are summarized in Figs. 4 and 5.

D_2-like receptor saturation binding - cocaine extinction training

For animals trained to self-administer cocaine which underwent extinction training and those given yoked cocaine/saline, an one-way ANOVA did not reveal a significant change in [^{3}H] raclopride binding characteristics: B_{max} (prefrontal cortex: $F(212) = 0.54$, $p = 0.60$; dorsal striatum $F(212)=1.54$, $p = 0.25$;

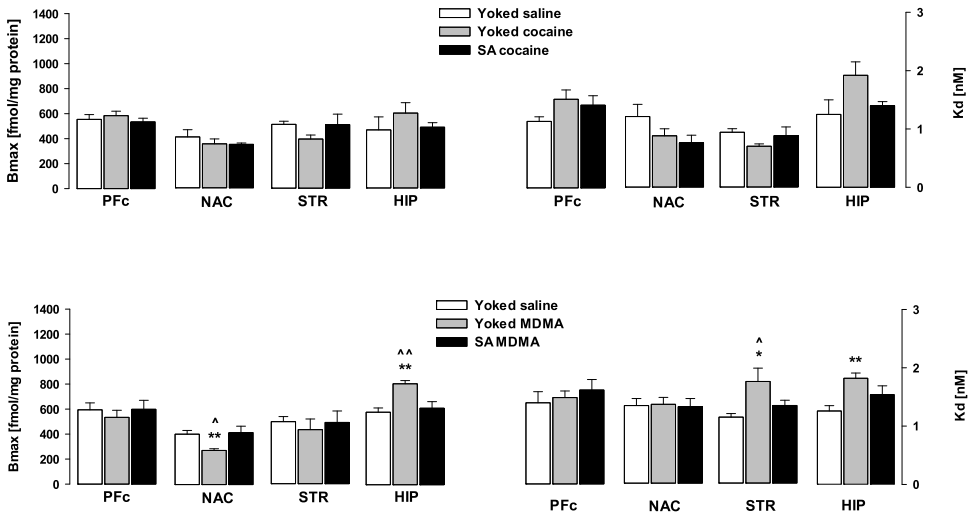

Fig. 4. The effects of cocaine (0.5 mg/kg/infusion; *top panels*) or MDMA (0.5 mg/kg/infusion; *bottom panels*) self-administration and the extinction training on the dopamine D_2-like receptor B_{max} and K_d values in the prefrontal cortex (PFc), nucleus accumbens (NAC), dorsal striatum (STR) and hippocampus (HIP). Control cocaine, MDMA and saline groups were generated by using 'yoked' procedure. Saturation biochemical binding analysis was performed with [^{3}H]raclopride. Values are the means (±SEM). N = 5 rats/group. $^*p < 0.05$; $^{**}p < 0.01$ *versus* corresponding yoked saline; $\hat{}p < 0.05$; $\hat{}\hat{}p < 0.01$ *versus* MDMA self-administration (SA MDMA).

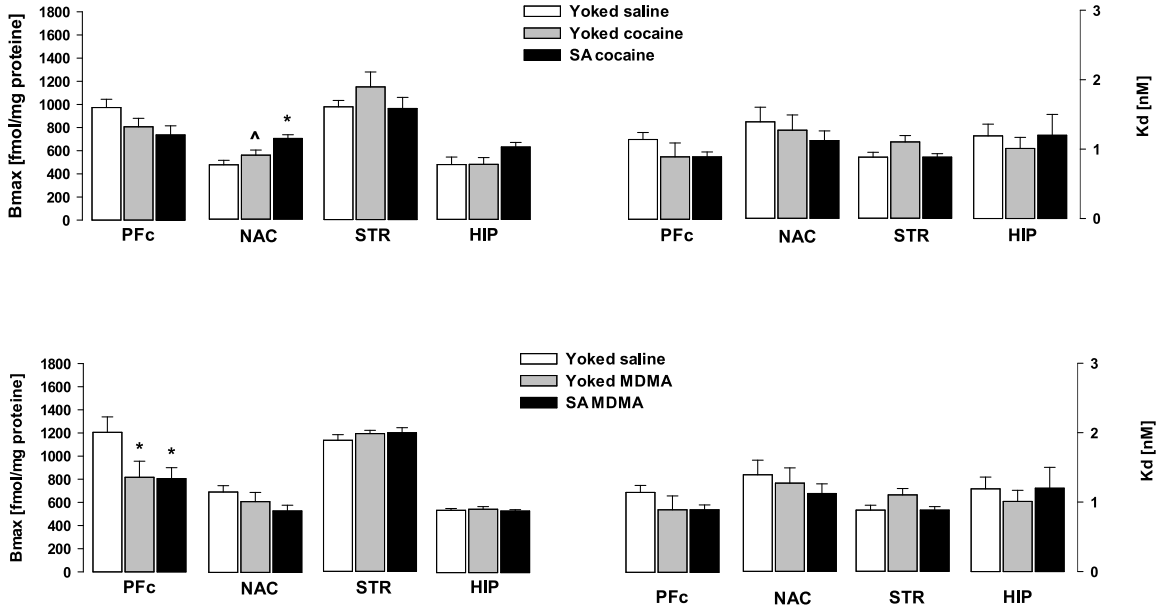

Fig. 5. The effects of cocaine (0.5 mg/kg/infusion; *top panels*) or MDMA (0.5 mg/kg/infusion; *bottom panels*) and the extinction training on the glutamate $mGlu_5$ receptor B_{max} and K_d values in the prefrontal cortex (PFc), nucleus accumbens (NAC), dorsal striatum (STR) and hippocampus (HIP). Control cocaine, MDMA and saline groups were generated by using 'yoked' procedure. Saturation biochemical binding analysis was performed with [^{3}H]MPEP. Values are the means (±SEM). N = 5 rats/group. $^*p < 0.05$ *versus* corresponding yoked saline; $\hat{}p < 0.05$; *versus* cocaine self-administration (SA cocaine).

hippocampus: F(212)=0.82, $p = 0.46$) and K_d (an one-way ANOVA: prefrontal cortex: F(212) = 2.08, $p = 0.17$; dorsal striatum F(212) =1.79, $p = 0.21$; hippocampus: F(212)=3.05, $p = 0.08$) in the rat brain structures. A non-significant trend to attenuate the D_2-like receptor B_{max} values was observed in the nucleus accumbens in both cocaine groups compared with yoked saline (F(212) = 3.40, $p = 0.06$), without changes in K_d (F(212) = 2.49, $p = 0.12$) (Fig. 4 top panels).

D_2-like saturation binding - MDMA extinction training

Extinction training in rats resulted in significant changes in [^{3}H] raclopride binding in the nucleus accumbens as shown by a significant alteration in the B_{max} values (an one-way ANOVA: F(212) = 7.22, $p < 0.01$), but not in the K_d values (F(212) = 0.03, $p = 0.97$) (Fig. 4 bottom panels). The Newman–Keuls' *post hoc* demonstrated a significant reduction in the D_2-like receptor B_{max} in rats treated passively with the drug *versus* self-administered cocaine ($p < 0.05$) and yoked saline ($p < 0.01$) groups. In the same animals, a significantly change in the B_{max} and K_d values was found also in the hippocampus (an one-way ANOVA B_{max}: F(212) = 14.10, $p < 0.001$, K_d: F(212) = 8.11, $p < 0.001$). The *post hoc* analysis revealed a significant rise in the D_2-like receptor B_{max} in rats treated passively with the drug ($p < 0.01$) *versus* self-administered cocaine and yoked saline groups. During extinction in experimental cage, the animals previously trained to self-administer MDMA and those given yoked MDMA did not show changes in the density

of D_2-like receptors in the dorsal striatum ($F(212) = 0.28$, $p = 0.76$), however, an one-way ANOVA indicated a significant increase of the receptor affinity in the yoked MDMA group ($F(212)=5.92$, $p < 0.05$). A non-significant change in the B_{max} ($F(212) = 0.37$, $p = 0.70$) as well as K_d values ($F(212) = 0.46$, $p = 0.64$) was observed in the prefrontal cortex (Fig. 4 bottom panels).

$mGlu_5$ receptor saturation binding - cocaine extinction training

In animals that were withdrawn from cocaine intake under extinction training protocol, an one-way ANOVA showed a significant effect on the $mGlu_5$ receptor density ($F(212) = 4.25$, $p < 0.05$), but not on the affinity ($F(212)=0.52$; $p = 0.61$) (Fig. 5 top panels) in the nucleus accumbens. The *post hoc* test revealed that the $mGlu_5$ receptor B_{max} was significantly elevated in self-administered cocaine group compared with the yoked saline and cocaine groups in this brain area ($p < 0.05$). A non-significant trend toward a decrease in $mGlu_5$ receptor density was also observed in the prefrontal cortex ($F(212) = 2.65$, $p = 0.11$) in both groups with a history of cocaine administration, while no changes were found for $mGlu_5$ receptor affinity ($F(212) = 1.01$; $p = 0.34$). In the dorsal striatum or hippocampus extinction training changed neither the B_{max} values ($F(212) = 1.36$, $p = 0.29$ and $F(212)=0.02$, $p = 0.98$, respectively) nor the K_d values ($F(212) = 0.04$; $p = 0.96$ and $F(212)=0.25$; $p = 0.78$; respectively) of [^{3}H]MPEP binding in animals with a history of cocaine self-administration and with yoked cocaine delivery compared with the yoked saline controls (Fig. 5 top panels).

$mGlu_5$ receptor saturation binding - MDMA extinction training

In animals undergoing extinction training during extinction from MDMA an one-way ANOVA showed a significant effect for the $mGlu_5$ receptor density ($F(212) = 3.37$, $p < 0.05$), but not for affinity $F(212)=1.40$, $p = 0.28$) (Fig. 5 bottom panels) in the prefrontal cortex. The *post hoc* analyses revealed a significant ($p < 0.05$) decrease (ca. 30%) in rats previously self-administering cocaine and those treated passively with the drug in comparison to yoked saline group in the prefrontal cortex ($p < 0.05$). As shown in Fig. 5 (bottom panels), in the other brain structures extinction training altered neither the B_{max} values (an one-way ANOVA: the nucleus accumbens $F(212) = 1.72$, $p = 0.22$; the dorsal striatum: $F(212)=0.76$, $p = 0.49$; the hippocampus: $F(212)=0.19$, p=0.83) nor the K_d values (the nucleus accumbens $F(212) = 1.57$, $p = 0.25$; the dorsal striatum: $F(212)=1.10$, $p = 0.36$; the hippocampus: $F(212)=0.35$, $p = 0.71$).

Discussion

In line with previous observations [24,25,28–32], a high rate of active lever pressing to receive cocaine or MDMA versus the inactive lever was observed in the maintenance phase. The main difference between cocaine and MDMA was that the latter drug evoked lower response rates and self-administration acquisition was slower. These observations on MDMA self-administration follow previous results of others authors [24,25]. It should also be mentioned that although all animals had been self-administering cocaine and MDMA, the number of injection during MDMA self-administration session were lower than during cocaine self-administration session. Significant different between cocaine and MDMA came from: (1) alternative mechanism of action to increase the level monoamines from their respective axon terminals (see Introduction [10–17]); (2) both drugs cause long-lasting suppression of dopamine and 5-HT neurons, although MDMA alters twice more on 5-HT neurons than on dopamine [5,13], (3) MDMA requires much longer time (few minutes), as compared to cocaine (few seconds) to initiate suppression of 5-HT and dopamine neuron activity, but the effect of suppression can be prolonged till 6 h, while in cocaine till 2 h [33]. Weaker responses MDMA on dopamine neurons, associated with drug-induced reinforcing property, produces much weaker drug dependence than cocaine (long time of acquisition, a larger percentage of non-dependent animals [present data, 5,24,25]), while stronger responses on 5-HT neurons, associated with drug-induced euphoria property, produces strong but extended effect correlated with amount of drug necessary to induces the effect (the number of injection during self-administration session, [present data, 5,24,25]). Replacement of cocaine or MDMA with vehicle and removal of drug-associated stimulus during extinction training caused reduction of frequency of active lever presses to 25% of that seen in the drug maintenance phase, and these findings have also been previously shown by us and other authors [24,25,28–31]. During reinstatement tests, cocaine or MDMA seeking behavior was produced by either exposure to the drug-associated cues or drug-priming injections. In our study, re-exposure to the stimulus previously associated with self-administered drugs enhanced only active lever presses. Moreover, the increase in responding following cocaine at a dose of 10 mg/kg and MDMA at a dose of 7.5 mg/kg was restricted to the active lever responding, so the presented data show specific effect, and drugs at those doses did not produce changes in inactive lever presses.

The extinction training does not protect from drug relapse however, it should be pointed that the reinstatement after extinction training and exposure to several contexts is significantly reduced as compared to reinstatement following withdrawal in home cage environments with social isolation (one of the most stressful conditions responses for cease abstinent and favoring relapse) for an equivalent amount of time [23,34,35]. It is also known that different types of priming stimuli engage separate neural substrates following extinction training, e.g., the nucleus accumbens and ventral hippocampus are involved in cocaine-induced drug seeking, but not in cue-induced reinstatement [35,36], while the basolateral region of the amygdala, the dorsomedial prefrontal cortex or the dorsal hippocampus are key regions for cue (but not drug) reinforcement [35]. Based on the above-mentioned findings, it was proposed that the effects of extinction training on these various forms of reinstatement could also be linked to different mechanisms during the extinction learning process. In this paper, we found that extinction training after cocaine self-administration and MDMA self-administration regulates the dopamine D_2-like and glutamate $mGlu_5$ receptor binding and affinity in the drug-dependent and brain area-dependent way. We observed that, cocaine extinction training did not alter significantly dopamine D_2-like receptor density or affinity the in the limbic and subcortical brain areas, however a non-significant attenuation was seen in the nucleus accumbens of rats with a history of drug self-administration. The same lack of changes in dopamine D_2-like receptor density and affinity were observed previously by our group [37] and other authors [38–40] in rats underwent cocaine self-administration and extinguished in experimental cage. These neurochemical data on the membrane receptors that display functional receptor population seem to be in line with our previous observation with the use of the same cocaine self-administration and extinction procedure. Thus, cocaine-experienced and saline control rats showed parallel similar basal concentrations of accumbal shell dopamine levels [41].

Only yoked MDMA administration and 10-day abstinence with extinction training generated significant changes in the dopamine D_2-like receptor binding and affinity in rats with a decrease in the nucleus accumbens (ca. 40%) and increase (by 28%) in the hippocampus for the dopamine D_2-like receptor B_{max} value and a rise (ca. 30%) in the striatum and hippocampus for the K_d value. The yoked procedure is traditionally intended to serve as a control to separate the biological effects of the drug from the effects that

may arise from self-administration behavior or from the impact of the drug on self-administration behavior. However, the yoked delivery of drug may even be aversive [42], and the observed changes in the binding parameters might be linked to some unpleasant stimulus. To support different neuromolecular mechanisms underlying various types of drug abstinence, recently we reported that housing conditions in rats withdrawn from cocaine self-administration evoke changes in the brain D_2-like receptor density. Thus, the home cage isolation significantly enhances the dopamine D_2-like receptor density in the hippocampus, while enriched environment or isolation reduces the receptor density in the dorsal striatum and enhances in the prefrontal cortex [23]. Altogether, the above discussed changes seem to be specific to the cocaine abstinence phase with the altered B_{max} and K_d values for dopamine D_2-like receptors in cocaine-experienced group of rats underwent isolation or enriched environment but not in rats exposed for extinction training.

Following extinction training in cocaine- or MDMA-treated rats, we found drug-dependent changes in the rat brain glutamate $mGlu_5$ receptor binding density but not affinity. In fact, both drugs change glutamate $mGlu_5$ receptor B_{max} value with significant effect observed for MDMA, however, such effects should be linked with pharmacological effects of any drug as they also generated parallel changes for yoked drug controls. Only for cocaine self-administration, we observed a 40% rise in the B_{max} value in the nucleus accumbens which is in agreement with the previous findings from our laboratory that cocaine self-administration and a 10-day drug-free period with extinction training reduced basal extracellular glutamate levels in the nucleus accumbens shell [41]. Such a reduction may contribute to the increase in glutamate $mGlu_5$ receptor density; however, the imbalance in glutamate transmission was not observed immediately after cocaine self-administration, as evidenced by both receptor binding and immunohistochemical staining procedures [27,43]. The present findings also support previous observations showing that 21-day extinction training after cocaine self-administration evokes a rise in glutamate $mGlu_5$ receptor protein expression in the accumbal core postsynaptic membrane fraction, while its surface expression is significantly reduced [44]. In should be underscored here that the above lack of close correspondence between changes in glutamate $mGlu_5$ receptors is due to the measurement of different receptor pools (the pool of cell surface membrane glutamate $mGlu_5$ receptors capable of binding the ligand and determination of the ligand affinity for the receptor using the radioactive ligand binding saturation method *vs.* the whole receptor protein pool, which involves also the inactive form due to internalization measured by Western blotting). The mechanism linked to the observed rise in $mGlu_5$ receptor density is likely associated with a new learning condition that occurs during extinction training. In fact, in this type of cocaine abstinence, animals learn that cocaine-seeking behavior in an environment previously associated with drug delivery (experimental cages) is no longer reinforced due to the absence of a drug reward. Glutamate $mGlu_5$ receptors are required for the consolidation of drug-related memories [45] and for increased $mGlu_5$ receptor signaling [46], whereas their inactivation hinders extinction learning and formation of new memory pathways [47], which could explain the $mGlu_5$ receptor up-regulation. Interestingly, in the prefrontal cortex a 25–30% reduction in the glutamate $mGlu_5$ receptor density in cocaine- or MDMA-abstinent rats was demonstrated, with significant effects being observed after previous MDMA exposure. Several lines of evidence indicate that the prefrontal cortex is critical for drug-seeking behavior [48], and several conditions herein even had beneficial effects by reducing drug relapse. Reductions of the glutamate $mGlu_5$ receptor protein and the postsynaptic density of these receptors were observed in the dorsomedial prefrontal cortex in rats following cocaine self-administration and extinction conditions [49] and in cocaine abstinent animals exposed to the experimental cage with lever retraction, while no changes in glutamate $mGlu_5$ receptor protein and mRNA were detected in rats that were placed in the home cage (social isolation) during abstinence [49,50]. In contrast to the latter authors, we recently found that the glutamate $mGlu_5$ receptor density decreased in the prefrontal cortex after different types of cocaine abstinence (isolation cage and enriched environment) [23] what together with the present data means that any type of housing conditions causes similar neuromolecular mechanisms in psychostimulant-related abstinence in rats. These changes may contribute to the reinstatement processes.

It should be underlined that the present study had some limitations as we did not separate the prefrontal cortex sub-areas that either drive expression (the prelimbic cortex) or suppression (the infralimbic cortex) of drug seeking behaviors [48,51,52].

In conclusions, this study for the first time shows how extinction training from cocaine or MDMA self-administration or passive-injections changes the dopamine D_2-like and glutamate $mGlu_5$ receptor B_{max} and/or K_d values in different regions of the rat brain.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

This research was supported by the Polish National Science Centre grant NCN Sonata2011/03/D/NZ7/06295 and the statutory funds of the Maj Institute of Pharmacology Polish Academy of Sciences, Kraków.

References

[1] Alterman AI, McDermott PA, Cook TG, Cacciola JS, McKay JR, McLellan AT, et al. Generalizability of the clinical dimensions of the addiction severity index to nonopioid-dependent patients. Psychol Addict Behav 2000;14:287–94.
[2] Pasareanu AR, Vederhus J-K, Opsal A, Kristensen Ø, Clausen T. Improved drug-use patterns at 6 months post-discharge from inpatient substance use disorder treatment: results from compulsorily and voluntarily admitted patients. BMC Health Serv Res 2016;16:291, doi:http://dx.doi.org/10.1186/s12913-016-1548-6.
[3] Koob GF. The neurobiology of addiction: a neuroadaptational view relevant for diagnosis. Addiction 2006;101(Suppl 1):23–30, doi:http://dx.doi.org/10.1111/j.1360-0443.2006.01586.x.
[4] Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 2001;24:97–129, doi:http://dx.doi.org/10.1016/S0893-133X(00)00195-0.
[5] Nutt DJ. The neurochemistry of addiction. Hum Psychopharm Clin 1997;12:53–8.
[6] Di Chiara G. The role of dopamine in drug abuse viewed from the perspective of its role in motivation. Drug Alcohol Depend 1995;38:95–137.
[7] D'Souza MS. Glutamatergic transmission in drug reward: implications for drug addiction. Front Neurosci 2015;9:404, doi:http://dx.doi.org/10.3389/fnins.2015.00404.
[8] Spencer S, Kalivas PW. Glutamate transport: a New bench to bedside mechanism for treating drug abuse. Int J Neuropsychopharmacol 2017;20:797–812, doi:http://dx.doi.org/10.1093/ijnp/pyx050.
[9] Wydra K, Gołembiowska K, Suder A, Kamińska K, Fuxe K, Filip M. On the role of adenosine (a) 2A receptors in cocaine-induced reward: a pharmacological and neurochemical analysis in rats. Psychopharmacology (Berl) 2015;232:421–35, doi:http://dx.doi.org/10.1007/s00213-014-3675-2.
[10] Bennett BA, Wichems CH, Hollingsworth CK, Davies HM, Thornley C, Sexton T, et al. Novel 2-substituted cocaine analogs: uptake and ligand binding studies at dopamine, serotonin and norepinephrine transport sites in the rat brain. J Pharmacol Exp Ther 1995;272:1176–86.
[11] Nestler EJ. The neurobiology of cocaine addiction. Sci Pract Perspect 2005;3:4–10.
[12] Bowyer JF, Young JF, Slikker W, Itzak Y, Mayorga AJ, Newport GD, et al. Plasma levels of parent compound and metabolites after doses of either d-fenfluramine or d-3,4-methylenedioxymethamphetamine (MDMA) that

produce long-term serotonergic alterations. Neurotoxicology 2003;24:379–90, doi:http://dx.doi.org/10.1016/S0161-813X(03)00030-5.
[13] Green AR, Mechan AO, Elliott JM, O'shea E, Colado MI. The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy"). Pharmacol Rev 2003;55:463–508, doi:http://dx.doi.org/10.1124/pr.55.3.3.
[14] McCann U, Szabo Z, Scheffel U, Dannals R, Ricaurte G. Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. Lancet 1998;352:1433–7, doi:http://dx.doi.org/10.1016/S0140-6736(98)04329-3.
[15] Battaglia G, Yeh SY, De Souza EB. MDMA-induced neurotoxicity: parameters of degeneration and recovery of brain serotonin neurons. Pharmacol Biochem Behav 1988;29:269–74, doi:http://dx.doi.org/10.1016/0091-3057(88)90155-4.
[16] Steele TD, Nichols DE, Yim GKW. Stereochemical effects of 3,4-methylenedioxymethamphetamine (MDMA) and related amphetamine derivatives on inhibition of uptake of [3H]monoamines into synaptosomes from different regions of rat brain. Biochem Pharmacol 1987;36:2297–303, doi:http://dx.doi.org/10.1016/0006-2952(87)90594-6.
[17] Rothman RB, Baumann MH, Dersch CM, Romero DV, Rice KC, Carroll FI, et al. Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. Synapse 2001;39:32–41, doi:http://dx.doi.org/10.1002/1098-2396(20010101)39:1<32::AID-SYN5>3.0.CO;2-3.
[18] Volkow ND, Fowler JS, Wang GJ, Baler R, Telang F. Imaging dopamine's role in drug abuse and addiction. Neuropharmacology 2009;56(Suppl 1):3–8, doi:http://dx.doi.org/10.1016/j.neuropharm.2008.05.022.
[19] Gould RW, Porrino LJ, Nader MA. Nonhuman primate models of addiction and PET imaging: dopamine system dysregulation. Curr Top Behav Neurosci 2012;11:25–44, doi:http://dx.doi.org/10.1007/7854_2011_168.
[20] Brown RM, Mustafa S, Akli Ayoub M, Dodd PR, G Pfleger KD, Lawrence AJ, et al. mGlu5 receptor functional interactions and addiction. Front Pharmacol 2012;3:84, doi:http://dx.doi.org/10.3389/fphar.2012.00084.
[21] Chiamulera C, Epping-Jordan MP, Zocchi A, Marcon C, Cottiny C, Tacconi S, et al. Reinforcing and locomotor stimulant effects of cocaine are absent in mGluR5 null mutant mice. Nat Neurosci 2001;4:873–4, doi:http://dx.doi.org/10.1038/nn0901-873.
[22] Cabello N, Gandía J, Bertarelli DCG, Watanabe M, Lluís C, Franco R, et al. Metabotropic glutamate type 5, dopamine d 2 and adenosine a 2a receptors form higher-order oligomers in living cells NIH public access. J Neurochem 2009;109:1497–507, doi:http://dx.doi.org/10.1111/j.1471-4159.2009.06078.x.
[23] Frankowska M, Miszkiel J, Pomierny-Chamioło L, Pomierny B, Giannotti G, Suder A, et al. Alternation in dopamine D $_2$ -like and metabotropic glutamate type 5 receptor density caused by differing housing conditions during abstinence from cocaine self-administration in rats. J Psychopharmacol 2019;33:372–82, doi:http://dx.doi.org/10.1177/0269881118821113.
[24] Schenk S, Gittings D, Johnstone M, Daniela E. Development, maintenance and temporal pattern of self-administration maintained by ecstasy (MDMA) in rats. Psychopharmacology (Berl) 2003;169:21–7, doi:http://dx.doi.org/10.1007/s00213-003-1407-0.
[25] Daniela E, Brennan K, Gittings D, Hely L, Schenk S. Effect of SCH 23390 on (F)-3,4-methylenedioxymethamphetamine hyperactivity and self-administration in rats. Pharmacol Biochem Behav 2004;77:745–50, doi:http://dx.doi.org/10.1016/j.pbb.2004.01.008.
[26] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 4th ed. Cambridge MA: Academic Press; 1998.
[27] Pomierny-Chamiolo L, Miszkiel J, Frankowska M, Bystrowska B, Filip M. Cocaine self-administration, extinction training and drug-induced relapse change metabotropic glutamate mGlu5 receptors expression: evidence from radioligand binding and immunohistochemistry assays. Brain Res 2017;1655:66–76, doi:http://dx.doi.org/10.1016/j.brainres.2016.11.014.
[28] Filip M, Faron-Górecka A, Kuśmider M, Gołda A, Frankowska M, Dziedzicka-Wasylewska M. Alterations in BDNF and trkB mRNAs following acute or sensitizing cocaine treatments and withdrawal. Brain Res 2006;1071:218–25, doi:http://dx.doi.org/10.1016/j.brainres.2005.11.099.
[29] Filip M, Frankowska M, Przegaliński E. Effects of GABA B receptor antagonist, agonists and allosteric positive modulator on the cocaine-induced self-administration and drug discrimination. Eur J Pharmacol 2007;574:148–57, doi:http://dx.doi.org/10.1016/j.ejphar.2007.07.048.
[30] Filip M, Frankowska M. Effects of GABA B receptor agents on cocaine priming, discrete contextual cue and food induced relapses. Eur J Pharmacol 2007;571:166–73, doi:http://dx.doi.org/10.1016/j.ejphar.2007.05.069.
[31] Schenk S, Hely L, Gittings D, Lake B, Daniela E. Effects of priming injections of MDMA and cocaine on reinstatement of MDMA-and cocaine-seeking in rats. Drug Alcohol Depend 2008;96:249–55, doi:http://dx.doi.org/10.1016/j.drugalcdep.2008.03.014.
[32] Nawata Y, Kitaichi K, Yamamoto T. Prevention of drug priming- and cue-induced reinstatement of MDMA-seeking behaviors by the CB1 cannabinoid receptor antagonist AM251. Drug Alcohol Depend 2016;160:76–81, doi:http://dx.doi.org/10.1016/j.drugalcdep.2015.12.016.
[33] Wei C, Han X, Weng D, Feng Q, Qi X, Li J, et al. Response dynamics of midbrain dopamine neurons and serotonin neurons to heroin, nicotine, cocaine, and MDMA. Cell Discov 2018;4:60, doi:http://dx.doi.org/10.1038/s41421-018-0060-z.
[34] Kelamangalath L, Swant J, Stramiello M, Wagner JJ. The effects of extinction training in reducing the reinstatement of drug-seeking behavior: involvement of NMDA receptors. Behav Brain Res 2007;185:119–28, doi:http://dx.doi.org/10.1016/j.bbr.2007.08.001.
[35] Bossert JM, Marchant NJ, Calu DJ, Shaham Y. The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research. Psychopharmacology (Berl) 2013;229:453–76, doi:http://dx.doi.org/10.1007/s00213-013-3120-y.
[36] Grimm JW, Hope BT, Wise RA, Shaham Y. Incubation of cocaine craving after withdrawal NIH public access. Nature 2001;412:141–2, doi:http://dx.doi.org/10.1038/35084134.
[37] Frankowska M, Marcellino D, Adamczyk P, Filip M, Fuxe K. Effects of cocaine self-administration and extinction on D2 -like and A2A receptor recognition and D2 -like/Gi protein coupling in rat striatum. Addict Biol 2013;18:455–66, doi:http://dx.doi.org/10.1111/j.1369-1600.2012.00452.x.
[38] Briand LA, Flagel SB, Garcia-Fuster MJ, Watson SJ, Akil H, Sarter M, et al. Persistent alterations in cognitive function and prefrontal dopamine D_2 receptors following extended, but not limited, access to self-administered cocaine. Neuropsychopharmacology 2008;33:2969–80, doi:http://dx.doi.org/10.1038/npp.2008.18.
[39] Briand LA, Flagel SB, Seeman P, Robinson TE. Cocaine self-administration produces a persistent increase in dopamine D2 high receptors. Eur Neuropsychopharmacol 2008;18:551–6, doi:http://dx.doi.org/10.1016/j.euroneuro.2008.01.002.
[40] Wallace DR, Mactutus CF, Booze RM. Repeated intravenous cocaine administration: locomotor activity and dopamine D2/D3 receptors. Synapse 1996;23:152–63, doi:http://dx.doi.org/10.1002/(SICI)1098-2396(199607)23:3<152::AID-SYN4>3.0.CO;2-7.
[41] Wydra K, Golembiowska K, Zaniewska M, Kamińska K, Ferraro L, Fuxe K, et al. Accumbal and pallidal dopamine, glutamate and GABA overflow during cocaine self-administration and its extinction in rats. Addict Biol 2013;18:307–24, doi:http://dx.doi.org/10.1111/adb.12031.
[42] Mutschler NH, Miczek KA. Withdrawal from a self-administered or non-contingent cocaine binge: differences in ultrasonic distress vocalizations in rats. Psychopharmacology (Berl) 1998;136:402–8.
[43] Pomierny-Chamiolo L, Miszkiel J, Frankowska M, Pomierny B, Niedzielska E, Smaga I, et al. Withdrawal from cocaine self-administration and yoked cocaine delivery dysregulates glutamatergic mGlu 5 and NMDA receptors in the rat brain fixed ratio DA dopamine NA norepinephrine 5-HT serotonin. Neurotox Res 2015;27:246–58, doi:http://dx.doi.org/10.1007/s12640-014-9502-z.
[44] Knackstedt LA, Schwendt M. mGlu5 receptors and relapse to cocaine-seeking: the role of receptor trafficking in postrelapse extinction learning deficits. Neural Plast 2016;9312508, doi:http://dx.doi.org/10.1155/2016/9312508.
[45] Kim JH, Perry C, Luikinga S, Zbukvic I, Brown RM, Lawrence AJ. Extinction of a cocaine-taking context that protects against drug-primed reinstatement is dependent on the metabotropic glutamate 5 receptor. Addict Biol 2015;20:482–9, doi:http://dx.doi.org/10.1111/adb.12142.
[46] Cleva RM, Olive MF. mGlu receptors and drug addiction. Wiley Interdiscip Rev Membr Transp Signal 2012;1:281–95.
[47] Andre MA, Gunturkun O, Manahan-Vaughan D. The metabotropic glutamate receptor, mGlu5, Is required for extinction learning that occurs in the absence of a context change. Hippocampus 2015;25:149–58, doi:http://dx.doi.org/10.1002/hipo.22359.
[48] Peters J, Kalivas PW, Quirk GJ. Extinction circuits for fear and addiction overlap in prefrontal cortex. Learn Mem 2009;16:279–88, doi:http://dx.doi.org/10.1101/lm.1041309.
[49] Behnam Ghasemzadeh M, Vasudevan P, Giles C, Purgianto A, Seubert C, Mantsch JR. Glutamatergic plasticity in medial prefrontal cortex and ventral tegmental area following extended-access cocaine self-administration. Brain Res 2011;1413:60–71, doi:http://dx.doi.org/10.1016/j.brainres.2011.06.041.
[50] Ben-Shahar O, Obara I, Ary AW, Ma N, Mangiardi MA, Medina RL, et al. Extended daily access to cocaine results in distinct alterations in homer 1b/c and NMDA receptor subunit expression within the medial prefrontal cortex. Psychopharmacology 2009;63:598–609, doi:http://dx.doi.org/10.1002/syn.20640.
[51] Goldstein RZ, Volkow ND. Dysfunction of the prefrontal cortex in addiction: neuroimaging findings and clinical implications. Nat Rev Neurosci 2011;12:652–69, doi:http://dx.doi.org/10.1038/nrn3119.
[52] Gass JT, Chandler LJ. The plasticity of extinction: contribution of the prefrontal cortex in treating addiction through inhibitory learning. Front Psychiatry 2013;4:46, doi:http://dx.doi.org/10.3389/fpsyt.2013.00046.