



Amphetamine Self-Administration and Its Extinction Alter the 5-HT_{1B} Receptor Protein Levels in Designated Structures of the Rat Brain

Joanna Miszkiel¹ · Joanna Jastrzębska¹ · Małgorzata Filip¹ · Edmund Przegaliński¹

Received: 5 June 2018 / Revised: 1 August 2018 / Accepted: 21 August 2018 / Published online: 30 August 2018
© The Author(s) 2018

Abstract

Manipulation of the serotonin (5-HT)_{1B} receptors can modify the behavioral effects of amphetamine including its reinforcing properties. Focus of this study was to examine changes in 5-HT_{1B} receptor protein expression in several brain structures linked to substance drug disorder in different stages of amphetamine addiction—single session of amphetamine self-administration, 20 consecutive days of amphetamine self-administration, and 3 and 14 days of extinction from chronic drug intake. “Yoked” procedure was employed to set apart pharmacological and motivational effects of amphetamine intoxication. Immunohistochemistry was performed on brain slices containing the following regions: nucleus accumbens (NAc) shell and core, globus pallidum (GP) lateral and ventral, hippocampus (HIP), substantia nigra (SN), and ventral tegmental area (VTA). Single amphetamine session decreased the amount of 5-HT_{1B} receptors in SN, VTA, and HIP in active and yoked rats. On the contrary, 20 days of chronic amphetamine exposure triggered elevation of 5-HT_{1B} receptors exclusively in animals that voluntarily administered the drug in NAc core, GP ventral, and HIP. Furthermore, 14-day (but not 3-day) extinction from amphetamine increased the 5-HT_{1B} receptor expression in ventral and lateral GP, HIP, and SN. This study is the first to demonstrate that exposure to amphetamine and its extinction alter the expression of 5-HT_{1B} receptors in various rat brain regions, and those changes seem to be transient and region specific. Importantly, since increased expression of 5-HT_{1B} receptor after chronic amphetamine self-administration was limited only to active group of animals, we suggest that 5-HT_{1B} receptor is linked to motivational aspect of addiction.

Keywords Serotonin (5-HT)_{1B} receptor expression · Immunohistochemistry · Amphetamine addiction · Amphetamine self-administration · Extinction training · Yoked procedure

Introduction

Serotonin (5-HT) transmission originates in raphe nuclei and spreads throughout the brain innervating almost all its parts (Parent et al. 1981; Steinbusch 1981). Abundance of the 5-HTergic connections, signaling via 14 different 5-HT receptors, makes this monoamine perfectly position to mediate many behavioral function, as well as to be responsible for maintaining a homeostasis of the system. Malfunction of this circuit can lead to many pathological states, among others,

depression, schizophrenia, autism, or substance use disorders (Hoyer et al. 2002; Filip and Bader 2009). In fact, several data showed that among 5-HT receptors, 5-HT_{1B} ones seem to be especially promising in addiction research. Being negatively coupled to adenylyl cyclase and classified as both pre- and postsynaptic auto- and heteroreceptors, 5-HT_{1B} receptors exert inhibitory role on the release of several neurotransmitters including glutamate, GABA, and dopamine (Adell et al. 2001; Hoyer et al. 2002; Sari 2004). Also, their presence (protein, mRNA, or both) was confirmed in the brain areas that are crucial to reward system (nucleus accumbens (NAc), ventral tegmental area (VTA), hippocampus (HIP), globus pallidus (GP)) as well as are associated with drug-induced hyperlocomotion (substantia nigra (SN)) (Pazos and Palacios 1985; Bruinvels et al. 1993; Sari et al. 1997, 1999). A part of the preferential location, several pharmacological data showed that they indeed play significant role in psychostimulant drug addiction such as amphetamine; namely, mice lacking the 5-

✉ Joanna Miszkiel
asia.miszkiel@gmail.com

¹ Department of Drug Addiction Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, 31-343 Kraków, Poland

HT_{1B} receptor exhibited increased sensitivity to amphetamine measured as increased in locomotor activity (Bronsert et al. 2001). Interestingly, when these receptors were pharmacologically stimulated, not only they enhanced amphetamine-induced hyperlocomotion (Papla et al. 2002) but also facilitated the development of sensitization (Przegaliński et al. 2001) and decreased the number of lever presses in fixed and progressive ratio schedule (Fletcher et al. 2002; Miszkiewicz et al. 2012) in amphetamine self-administration model. Moreover, in the latter animal paradigm, administration of 5-HT_{1B} receptors antagonist attenuated the amphetamine-evoked seeking behavior (Miszkiewicz et al. 2012). However, there is still little known about molecular background of the 5-HT_{1B} receptor involvement in stages of amphetamine addiction.

On the other hand, there is a small body of literature indicating that mentioned receptors undergo time-dependent bidirectional alternation during the abstinence from chronic self-administration of another psychostimulant—cocaine (O'Dell et al. 2006). Furthermore, quantitative autoradiography indicated that after 5-day withdrawal from subchronic cocaine administration, 5-HT_{1B} receptors were upregulated in numerous brain structures of rat (Przegaliński et al. 2003). Although amphetamine and cocaine belong to the same group of psychostimulant drugs, they display several discrepancies in regard to 5-HT_{1B} receptor mediated effects. For instance, pharmacological stimulation of 5-HT_{1B} receptors increases reinforcing effects of cocaine (Parsons et al. 1998; Filip et al. 2002; Przegaliński et al. 2007; Pentkowski et al. 2009, 2012) but not of amphetamine (Fletcher and Korth 1999; Fletcher et al. 2002; Miszkiewicz et al. 2012). Moreover, even though 5-HT_{1B} receptor blockade attenuates cocaine (Przegaliński et al. 2008) and amphetamine-seeking behavior (Miszkiewicz and Przegaliński 2013), activation of those receptors decreases or has no effect on reinstatement of cocaine (Pentkowski et al. 2014) and amphetamine (Miszkiewicz and Przegaliński 2013), respectively. Keeping above in mind, it seems crucial to further investigate and eventually clarify the role of 5-HT_{1B} receptors in the drug addiction cycle. Therefore, this study was designed to determine the potential alternations in 5-HT_{1B} receptor protein level across different aspects of amphetamine addiction in rats.

Materials and Methods

Animals

Male Wistar rats (250–270 g) delivered by the licensed breeders (Charles River, Germany) were used in this experiment. Animals were housed individually in standard plastic rodent cages in a room maintained at 21 ± 1 °C and 40–50% humidity under a 12-h light-dark cycle (lights on at 6.00 a.m.). Rats were water restricted during initial lever-pressing training

sessions and first 5 days of amphetamine self-administration. At other times, water was ad libitum. Access to food was unlimited throughout all experiment. All procedures were conducted during the light phase of the light-dark cycle. The experimental procedures were carried out in accordance with the European Directive 2010/63/EU and were approved by the Bioethical Committee at the Institute of Pharmacology, Polish Academy of Sciences, Krakow. Animals were drug-naive.

Behavioral Experiments

Drugs

D-amphetamine sulfate (Sigma-Aldrich, USA) was dissolved in sterile 0.9% NaCl and given intravenously (0.1 ml/infusion).

Initial Lever-Press Training and Catheter Implantation

After 7-day initial habituation period, animals were water deprived for 18 h and then trained to lever press in 2-h daily sessions under the fixed ratio schedule (FR1) of water reinforcement for 2 to 5 days. Two days following the lever-press training and free access to water, the rats were implanted with a silastic catheter in the external jugular vein, as described previously (Filip et al. 2005). After surgery, the catheters were flushed daily with 0.1 ml of a heparinized saline solution (70 U/ml, Biochemie, Austria) and 0.1 ml of a cephalosporin solution (10 mg/ml, Biochemie GmbH, Austria). No problems with catheter patency were reported in the tested rats. The rats were allowed 5 to 8 days to recover from surgery before the start of the experiments.

Maintenance of Amphetamine Self-Administration

After recovery, animals were again water restricted and placed in operant chambers to press lever for water for one 2-h session. Self-administration experiment begun on the following day, and rats were divided into separate groups ($n = 6$ each) and trained to press the lever for amphetamine reinforcement during 2-h daily sessions performed 6 days/week (for details see Miszkiewicz et al. 2012). Briefly, once placed in operant chamber, animals had to choose to press one of two presented levers. Each press (FR1) on the “active” lever resulted in a 5-s infusion of amphetamine (0.12 mg/kg/infusion), whereas pressing the “inactive” lever had no consequences. After 5 days during which animals reached a stable responding on the active lever, the number of presses that was required to achieve amphetamine injection was increased to FR3, and after subsequent 5 days, was increased again to FR5. Rats were allowed to remain on this schedule for 5 days to reach stable responding. Subsequently, amphetamine dose was reduced to 0.06 mg/kg/infusion, and self-administration was

continued under FR5 for another 5 days. During the final 5 days, the number of active lever presses and the number of received infusions of amphetamine were stable and were not different between sessions by more than 10%; the number of inactive levers was no more than 50%. The number of daily amphetamine infusions was at least 10.

The animals actively administering amphetamine and corresponding “yoked” amphetamine or saline groups (for “yoked” procedure see below) were sacrificed immediately following the final 20th amphetamine self-administration session (see Scheme 1).

Single Amphetamine Self-Administration Session

After convalescence period, another group of animals were water deprived and reintroduced to operant chambers to press lever for water for one session. Next day, rats were allowed to self-administer amphetamine in self-administration operant chambers for one 2-h session under FR1 schedule. Every active lever pressing resulted in a 5-s delivery of amphetamine (0.06 mg/kg/infusion), and inactive lever pressing had no effect.

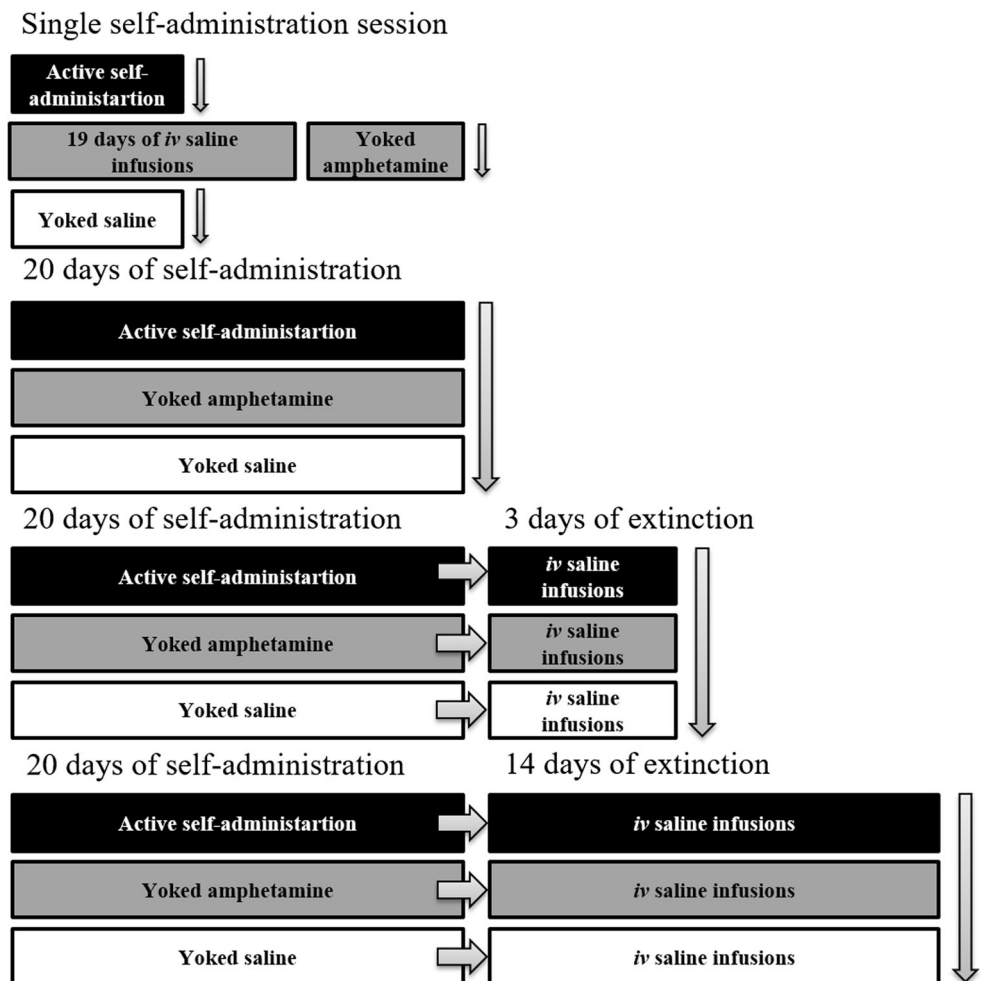
Animals actively administering amphetamine and corresponding “yoked” saline group were sacrificed immediately after the session (for “yoked” procedure see below). “Yoked” amphetamine rats were first introduced to 19 sessions during which they received passive saline injections in the same manner as active animal self-administered amphetamine. On the 20th final session, “yoked” amphetamine animals were passively administered amphetamine injections and were sacrificed instantly after.

Extinction Training

After 20 days of amphetamine self-administration, the rats underwent the extinction paradigm. During 2-h extinction session, animals were placed in the operant chambers described above and allowed to lever press (FR5); however, amphetamine was no longer present, and active lever presses resulted in saline delivery instead. Like in the maintenance, pressing the “inactive” lever had no consequences.

The animals actively administering amphetamine and corresponding “yoked” amphetamine or saline groups

Scheme 1 Experimental design for behavioral experiments. Vertical arrows represent time when appropriate group of animals were sacrificed



(for “yoked” procedure see below) were sacrificed upon completion of third or 14th extinction session (see Scheme 1).

“Yoked” Procedure

To distinguish between the pharmacological and motivational effects of amphetamine intake, “yoked” procedure was used for all experiments employed here. In this procedure, each rat actively self-administering amphetamine (or saline during extinction phase) has been assigned two rats that were passively receiving either amphetamine (or saline during extinction phase) or its vehicle in the same amount and manner as the active animal. Lever pressing by the “yoked” rats was recorded but had no programmed consequences.

Tissue Preparation

Immediately after the appropriate experimental sessions, animals ($n = 6$ group) were injected with pentobarbital (133.3 mg/kg, *i.p.*, Morbital, Biowet, Puławy, Poland) and perfused intracardially with a solution of 4% paraformaldehyde in 100-Mm phosphate buffer (pH = 7.4). The brains were immersed in the same fixative for 12 h. Then, tissues were permeated in 10% *w/v* sucrose at 4–8 °C for 7 days followed by 30% *w/v* sucrose for no less than 48 h.

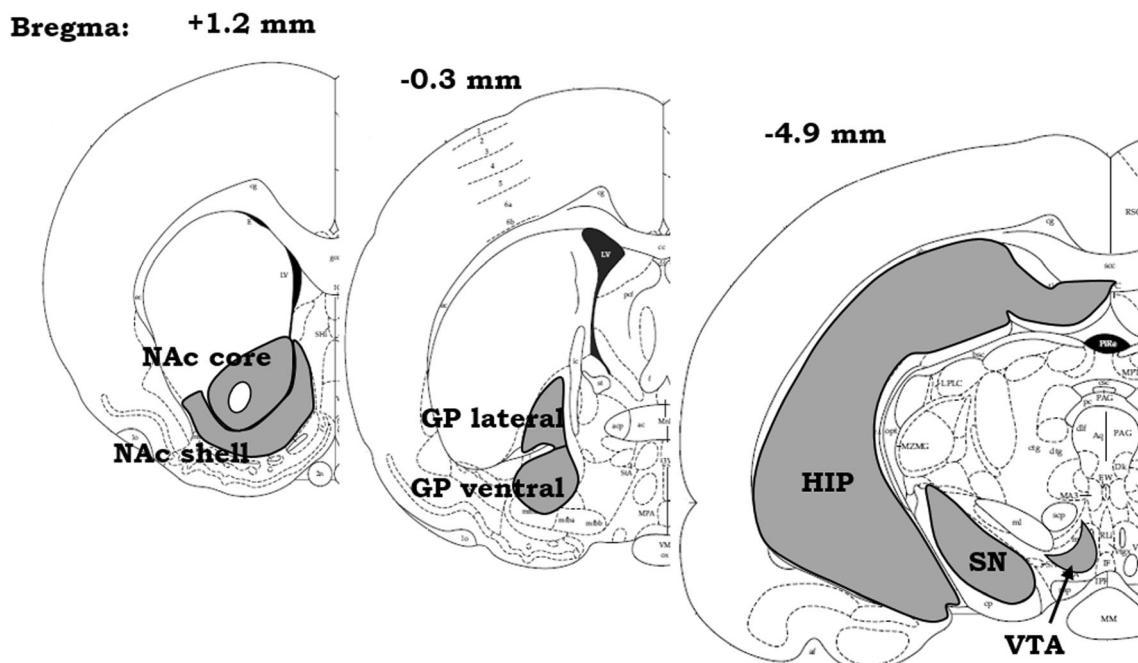
Immunohistochemistry Analyses

Brain Section Preparation

The brains were deeply frozen on dry ice, cut into 14- μ m coronal sections on a cryostat (Leica Microsystems, Nussloch, Germany), and kept at -22 °C until processed for immunohistochemistry. Stereotactic coordinates for the following brain structures: NAc shell and core, GP lateral and ventral, HIP, SN, and VTA, were determined and selected based on The Rat Brain Atlas (Paxinos and Watson 1998) (see Scheme 2). Brain slices with corresponding brain region(s) were mounted on gelled glass slides, in such a way that each slide housed all selected brain areas of one animal.

Protocol

Rat brain sections were rinsed with 100-mM PBS buffer (pH = 7.4) followed by the 30-min permeabilization in PBS buffer containing 0.1% Triton X-100 at room temperature. Afterwards, brain slices were incubated in Odyssey Blocking Buffer (OBB, Li-COR Biosciences, Cambridge, UK) for 1 h at room temperature. 5-HT_{1B} primary antibody (rabbit, polyclonal; Santa Cruz; sc-1460-R) was diluted to final concentration of 1:300 in OBB containing 0.1% Tween 20 and incubated on a specimen overnight at 4 °C. On the next day, brain slices were washed in PBS containing 0.1% Tween 20 (4 × 5 min), and secondary antibody goat anti-rabbit (IRDye® 680CW; Li-COR Biosciences, Cambridge, UK)



Scheme 2 Scheme represents coronal brain structures: nucleus accumbens shell (NAc shell) and core (NAc core), globus pallidum lateral (GP lateral) and ventral (GP ventral), hippocampus (HIP),

substantia nigra (SN), and ventral tegmental area (VTA) and their stereotactic coordinates according to the The Rat Brain Atlas (Paxinos and Watson 1998)

was applied and incubated at room temperature for 1 h. Slices were then washed in PBS containing 0.1% Tween 20 (4×5 min) and PBS (1×5 min) and left to dry. Fluorescence was detected using the Odyssey® Infrared Imaging System (21- μ m resolution, 1-mm offset with high quality) using 700-nm channel. The integrated intensities were determined with the associated Odyssey software. Each section was prescanned at different intensity settings on the Odyssey Classic Infrared Imaging System. Channel sensitivity was optimized for each set of stained sections, and channel intensity varied from 2 to 5. The latter allows detection of nonspecific background signals from the sample and permits gross localization of the cerebral tissue. Regions of interest were defined by comparison to The Rat Brain Atlas (Paxinos and Watson 1998) and marked using the in-software drawing tool. Data expressed as fluorescence relative units were later exported, analyzed, and normalized to saline-treated animals.

Statistical Analyses

Behavioral data were analyzed by a two-way analysis of variance (ANOVA) for repeated measures, whereas for the immunohistochemical assays, one-way ANOVA was used. If the effect was significant, a post hoc Newman-Keuls' test was applied to evaluate statistically significant differences between the treatment groups. All data are presented as the mean \pm SEM, and in all cases, p value less than 0.05 was considered significant.

Results

Behavioral Studies

Animals that were introduced to single 2-h amphetamine session scored 38.4 ± 9.4 lever presses on the active lever and 6.7 ± 3.1 on the inactive, receiving on average 1.6 ± 0.1 mg/kg amphetamine per rat.

Rats from remaining experiments that were allowed to self-administer amphetamine for 20 days showed stable level of response (FR5) during last five amphetamine sessions. Averaged number of active lever presses during the last 5 days of amphetamine (0.06 mg/kg) self-administration was 128 ± 11 on the active and 22 ± 1 on the inactive lever, while the number of infusions was 17 ± 2 . Throughout the 20 days of amphetamine self-administration, rats earned on averaged 33 ± 1.4 mg/kg amphetamine per rat. The two-way ANOVA for repeated measures showed that from the day 11 to the end of the maintenance phase, the number of active lever presses was statistically greater than the number of the inactive lever presses ($p < 0.05$; Fig. 1a–c). During the extinction phase, amphetamine was no longer available, which resulted in a decrease in active lever presses; and from the 21st to the 23rd

or 34th experimental sessions, the difference between responses to the active versus inactive lever was no longer significant (Fig. 1b, c, respectively).

In the yoked amphetamine groups, animals received the same amount of amphetamine at the same time as the animals that learned to self-inject amphetamine, without developing the preference towards active lever. Number of active lever presses did not differ from inactive lever presses neither during the maintenance nor the extinction phase (Fig. 1d, e) except the yoked amphetamine animals from the group that underwent 14 days of extinction. The latter group exhibited small, but yet significant ($p < 0.05$), increase in active lever presses from day 6 to day 12 (Fig. 1f). Rats in yoked saline groups received saline infusions in the same manner as paired active rat self-administer amphetamine. Except for the first day, the difference between responses to the active versus inactive lever was not significant (Fig. 1g–i).

Immunohistochemical Staining

The 5-HT_{1B} receptor protein expression was assessed in the following brain regions: NAc shell and core, GP dorsal and ventral, HIP, SN, and VTA. Protein expression was examined in four time points—after single 2-h amphetamine session, after 20 days of drug self-administration, and after 3 and 14 days of extinction from amphetamine.

Single 2-h Amphetamine Session

According to one-way ANOVA analysis, animals that underwent single 2-h amphetamine session showed a significant effect for 5-HT_{1B} receptor immunofluorescence in HIP ($F_{(2, 15)} = 19.735$; $p < 0.001$), SN ($F_{(2, 15)} = 28.590$; $p < 0.001$), and VTA ($F_{(2, 15)} = 4.826$; $p < 0.05$) (Fig. 2). Further post hoc analysis revealed that vast, up to 40%, decrease in 5-HT_{1B} receptor protein expressions in HIP and SN in both amphetamine-treated groups was significantly lower ($p < 0.001$) than protein levels of this receptor in yoked saline animals. Smaller (25%), but also significant, reduction in 5-HT_{1B} receptor expression was observed in active and yoked amphetamine animals ($p < 0.05$) in comparison to saline control group in VTA.

Chronic Amphetamine Self-Administration

The effect of amphetamine self-administration on 5-HT_{1B} receptor expression in rat brain structures is shown in Fig. 3. One-way ANOVA shown significant changes in NAc core ($F_{(2, 15)} = 9.109$; $p < 0.01$), lateral ($F_{(2, 15)} = 3.738$; $p < 0.05$), and ventral ($F_{(2, 15)} = 6.533$; $p < 0.01$) parts of GP, HIP ($F_{(2, 15)} = 4.119$; $p < 0.05$), and SN ($F_{(2, 15)} = 38.158$; $p < 0.001$). A similar but insignificant trend was observed for NAc shell ($F_{(2, 15)} = 3.030$; $p = 0.078$).

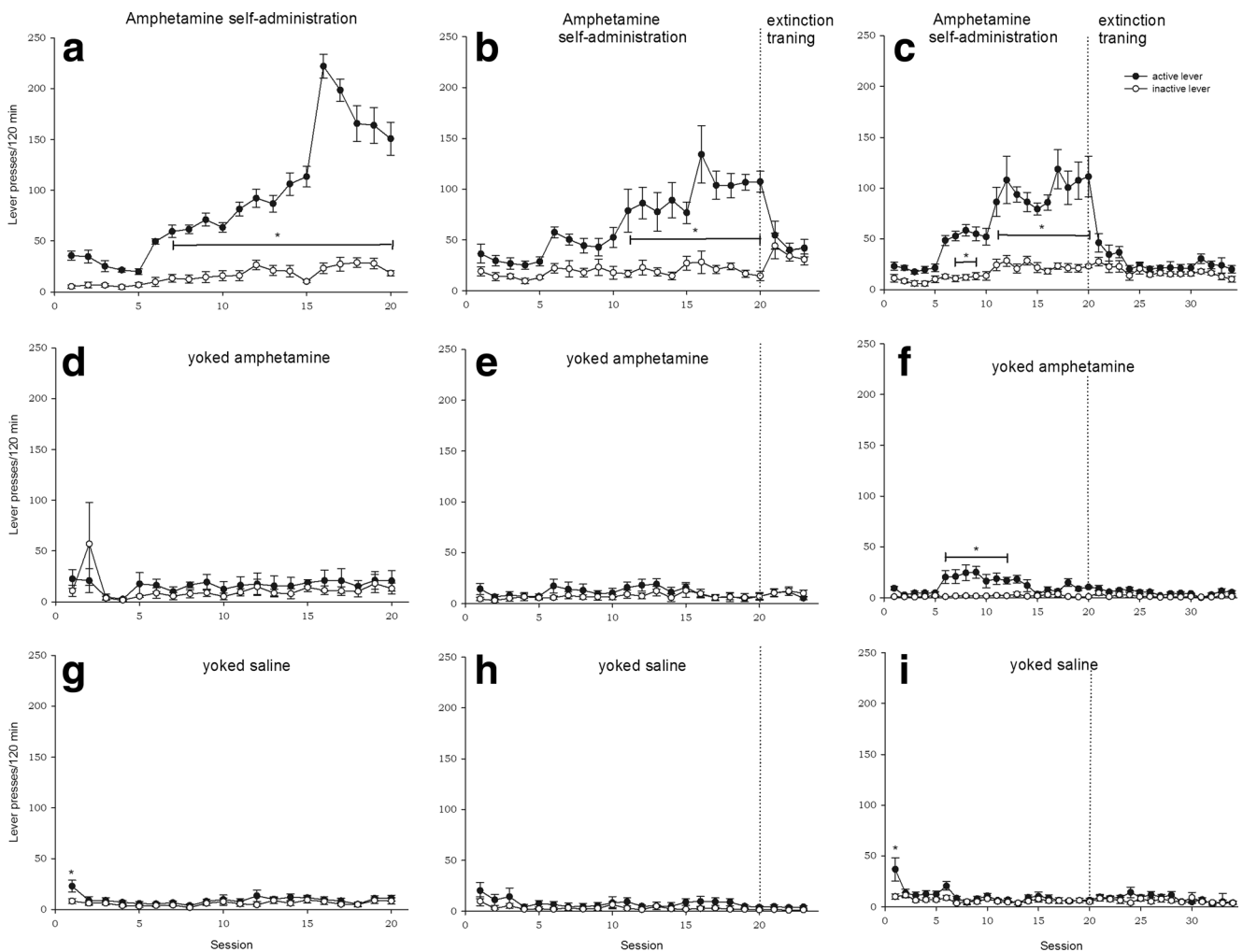


Fig. 1 The number of active and inactive lever presses in rats that acquired and maintained amphetamine (0.12–0.6 mg/kg/infusion) self-administration (**a**), 3-day extinction training (**b**), and 14-day extinction training (**c**) with their yoked controls that passively received

amphetamine (**d–f**) or saline (**g–i**). Data are presented as the mean \pm SEM from 6 rats/group. * indicates the statistically significant difference between active and inactive lever presses of minimum value $p < 0.05$

Newman-Keuls' post hoc analysis determined that in NAc core, level of the 5-HT_{1B} receptor protein in animals actively administering amphetamine was elevated (25%) and significantly different from levels found in yoked saline ($p < 0.05$) and amphetamine ($p < 0.01$) rats. A robust increase (55 and 80%) of examine receptors in animals with self-administration history versus yoked saline rats was also noted in in lateral and ventral parts of GP. However, according to the post hoc test employed, only the latter increase was significant ($p = 0.05$, $p < 0.01$, respectively). Moreover, this alternation was also significant in relation to the group of rats passively given the drug ($p < 0.05$). In the HIP, a significant ($p < 0.05$) 30% enhancement in immunofluorescence signal was seen in rats actively taking amphetamine in comparison to the yoked saline group. In SN increase in 5-HT_{1B} receptor expressions was observed in both active (55%; $p < 0.001$) and yoked amphetamine (30%; $p < 0.001$) rats versus control.

Moreover, post hoc test showed a significant difference between animals actively taking and passively receiving amphetamine injections ($p < 0.001$).

Short (3-Day) Extinction from Amphetamine Self-Administration

No changes in receptor expression were observed at this time point (Fig. 4).

Long (14-Day) Extinction from Amphetamine Self-Administration

As shown on Fig. 5, following 14-day extinction training, a one-way ANOVA showed a significant effect in 5-HT_{1B} receptor protein expressions in GP lateral ($F_{(2, 15)} = 36.095$, $p < 0.001$) and ventral (34%) ($F_{(2, 15)} = 23.819$; $p < 0.001$), HIP (25%) ($F_{(2, 15)} = 9.769$; $p < 0.01$), and SN ($F_{(2, 15)} =$

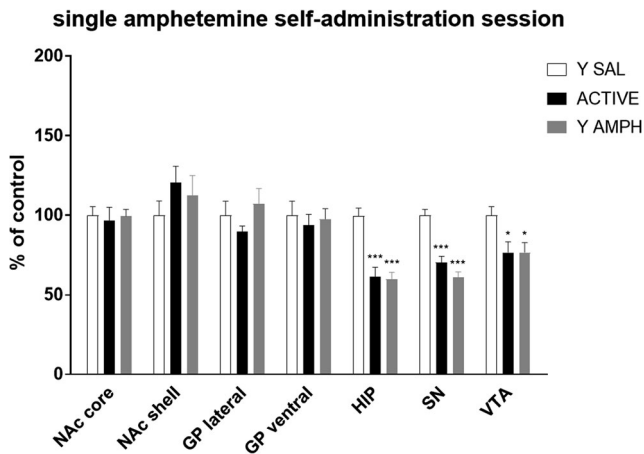


Fig. 2 Effect of single amphetamine self-administration session on the expression of 5-HT_{1B} receptors in nucleus accumbens shell (NAc shell) and core (NAc core), globus pallidum lateral (GP lateral) and ventral (GP ventral), hippocampus (HIP), substantia nigra (SN), and ventral tegmental area (VTA) in animals voluntarily taking the drug (ACTIVE). Control groups of rats passively receiving amphetamine (Y AMPH) or saline (Y SAL) were generated by “yoked” procedure. Data were normalized to saline-treated animals (% of yoked saline) and are shown as the mean (± SEM) of 6 subjects/group. Data were analyzed using one-way ANOVA and the post hoc Newman-Keuls’ test. **p* < 0.05 and ****p* < 0.001 versus yoked saline within that brain structure

13.482; *p* < 0.001) in rats with a history of amphetamine self-administration and their yoked amphetamine controls. After the post hoc test, significant (*p* < 0.001), 40% increase of the immunofluorescent signal was noted in animals with active and passive amphetamine intake history in lateral and ventral GP. Lower (25%) but significant (*p* < 0.01) rise in expression of 5-HT_{1B} receptors was observed in HIP also in both active and yoked amphetamine rats versus control group. The same trend was shown in SN, where 40 and 60% elevation in 5-HT_{1B} receptor protein was reported in rats previously

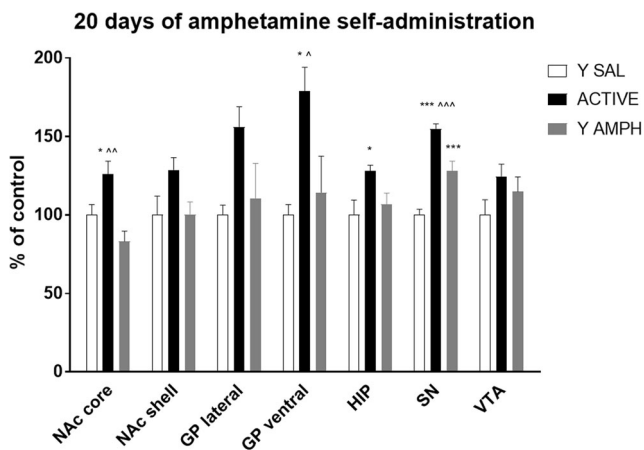


Fig. 3 Effect of 20-day amphetamine self-administration on the expression of 5-HT_{1B} receptors. For more details see Fig. 2. **p* < 0.05, ****p* < 0.001 versus yoked saline within that brain structure. ^*p* < 0.05, ^^*p* < 0.01, and ^^*p* < 0.001 versus yoked amphetamine within that brain structure

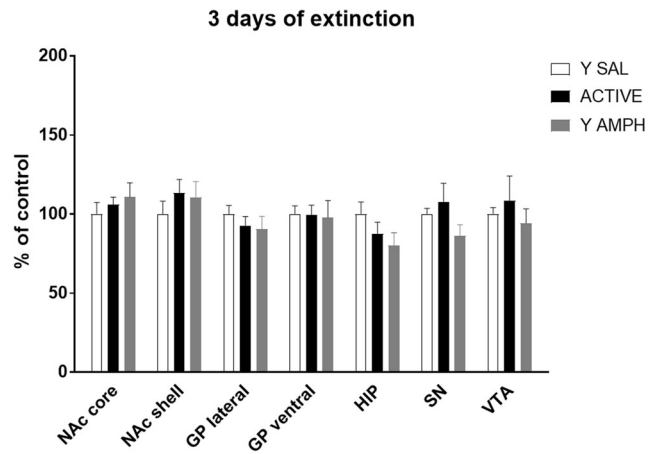


Fig. 4 Effect of 3-day extinction from chronic amphetamine self-administration on the expression of 5-HT_{1B} receptors. For more details see Fig. 2

voluntarily administered (*p* < 0.01) and passively received (*p* < 0.001) amphetamine, respectively.

Discussion

Classical theory of receptor regulation explains that G protein-coupled receptors (GPCRs) can undergo internalization and/or downregulation when overstimulated or becomes upregulated in response to long-term receptor blockade or deprivation. This theory has been supported for numerous receptors including those for 5-HT ones. In general, various 5-HT receptors, for instance 5-HT_{1B}, will downregulate when exposed to extensive 5-HT system activation (Fabre et al. 2000; van Oekelen et al. 2003) and upregulate when 5-HT neurotransmission system becomes impaired (Manrique et al. 1994; Compan et al. 1998).

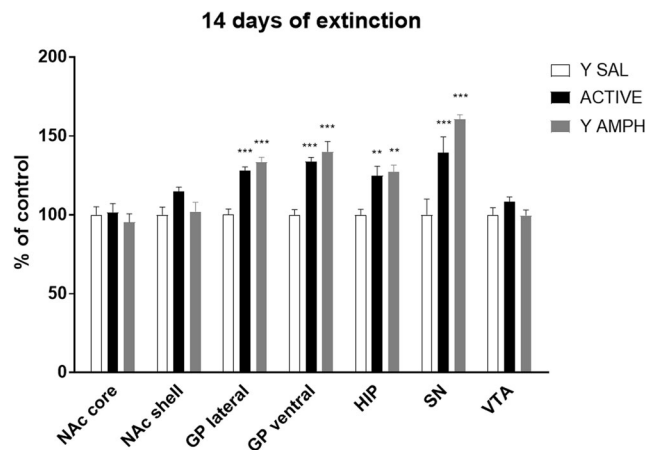


Fig. 5 Effect of 14-day extinction from chronic amphetamine self-administration on the expression of 5-HT_{1B} receptors. For more details see Fig. 2. ***p* < 0.01 and ****p* < 0.001 versus yoked saline within that brain structure

A strong psychostimulant—amphetamine—primarily known to elevate the extracellular levels of DA and NE, also elevates the 5-HT concentration (for review see Faraone 2018). Interestingly, however, it was shown that this drug can alter 5-HT efflux in both directions, depending on administration pattern, time, and brain structure. Indeed, single amphetamine administration increased extracellular efflux of 5-HT in various brain structures (Parada et al. 1988; Kuczenski and Segal 1997; Salomon et al. 2006; Pum et al. 2007), but when this drug was administered repeatedly, global 5-HT concentration declined (McMillen et al. 1991), while local (for instance PFCx) levels of this monoamine remained intact (Salomon et al. 2006). Furthermore, when extracellular 5-HT was measured after withdrawal from chronic amphetamine treatment, Barr et al. (2010) reported initial decrease (measured 20 h after last injection) in 5-HT concentration in dentate gyrus that was no longer detected after 4 weeks of drug-free period. Interestingly, 4 days after repeated amphetamine administration, increased cortical extracellular 5-HT level was reported following p-chloroamphetamine administration (Salomon et al. 2006).

In light of the theory of receptor regulation, these 5-HT fluctuations in response to amphetamine administration may lead to adaptations on the receptor protein level. Indeed, our study demonstrated that this psychostimulant triggered changes in the 5-HT_{1B} receptor density that are similar to the amphetamine-induced changes in extracellular 5-HT described above and that alternations were dynamic, time, and structure dependent. To the best of our knowledge, there are no other studies regarding changes in 5-HT_{1B} receptor density following amphetamine administration that employed self-administration model. However, Bonhomme et al. (1995) using sensitization paradigm and quantitative autoradiographic analysis examined the expression of 5-HT_{1B} receptor changes in several brain structures on the days 1 and 15 after 6-day experimenter-administered amphetamine regime. Similar to our results obtained from yoked rats, they showed no changes in 5-HT_{1B} receptor expression levels in medial PFCx, NAc, STR, SN, VTA, and medial raphe nuclei shortly after chronic amphetamine exposure. Interestingly, no changes were detected 2 weeks later, whereas we found an increase in 5-HT_{1B} protein levels at this time point. One possible explanation of the lack of receptor upregulation in sensitized animals is the difference in the housing condition that, in case of Bonhomme et al. study (1995), could have helped elevate the amphetamine withdrawal related stress/depression. Specifically, rats from our experiments were isolated throughout entire self-administration/extinction procedure, whereas animals from Bonhomme et al. (1995) studies were placed into individual cages only for the duration of the behavioral scoring. Lack of drug in the system due to the forced withdrawal period in both of the experiments most likely triggered the anxiety and depression-like states in all animals (Barr et al.

2010; Vuong et al. 2010; Li et al. 2014; Reinbold et al. 2014; Tu et al. 2014); however, the amphetamine-related withdrawal symptoms could have been less severe in animals which had contact with their littermates for the majority of time. In fact, recent study has shown that those drug-evoked withdrawal symptoms and depressive-like behaviors can be diminished when animals are provided with environmental enrichment (Hajheidari et al. 2015). Even though the housing condition employed by Bonhomme et al. (1995) do not resemble the environmental enrichment habitats per se, it is likely that it was enough to counterbalance the 5-HT_{1B} receptor upregulation observed in our experiment.

Another explanation to the elevated expression of the 5-HT_{1B} receptors after 2 weeks of amphetamine-free period could be that it was a result of their contribution to the incubation of craving (Pickens et al. 2011). Indeed, research has shown that seeking behavior increases in time-dependent manner during the forced abstinence from drug of abuse, including psychostimulants such as cocaine (Grimm et al. 2001; Mead et al. 2007; Neisewander et al. 2000) and methamphetamine (Li et al. 2015; Venniro et al. 2017). Moreover, at least regarding to cocaine, this phenomenon was reported to be partially driven by 5-HT_{2C} receptors (Swinford-Jackson et al. 2016). There is no evidence yet linking 5-HT_{1B} receptors with incubation of amphetamine craving; however, study showed that stimulation of this receptor attenuates cocaine-seeking behavior in a manner depended on length of abstinence (Pentkowski et al. 2014). With this in mind, the possibility that observed increase in 5-HT_{1B} expression protein in our study was related to incubation of amphetamine craving cannot be ruled out. Whether that lack of such increase reported by Bonhomme et al. (1995) was a consequence of the housing conditions or the passive drug administration needs to be determined.

As mentioned above, there is very little known about the effect of amphetamine on 5-HT_{1B} receptors; however, a few studies examined the effect of its derivative—3,4-methylenedioxymethamphetamine (MDMA) on these receptors. These studies showed that after the last dose of intermittent MDMA treatment, 5-HT_{1B} receptor mRNA was increased in several brain structures including STR (Kindlundh-Högberg et al. 2006), but that increase was undetected 24 h following the chronic drug administration (Sexton et al. 1999). Interestingly, at the latter time point, an increase in 5-HT_{1B} receptor binding sites in the STR was reported, regardless unchanged mRNA levels (Sexton et al. 1999). Transient and unequivocal alternations in levels of 5-HT_{1B} mRNA receptors were also observed after administration of another psychostimulant—cocaine. Acute administration of this drug had either no effect (Hoplight et al. 2007) or increase in the 5-HT_{1B} mRNA levels in STR (Neumaier et al. 2009). Passive chronic cocaine administration elevated 5-HT_{1B} mRNA (Hoplight et al. 2007), but when animals were self-administering the same drug, it had no effect on 5-HT_{1B}

receptor levels (Neumaier et al. 2009). Furthermore, 5-day withdrawal from chronic passive cocaine exposure led to increased 5-HT_{1B} receptor levels in several brain structures including NAc shell and STR (Przegaliński et al. 2003), whereas 2-week forced abstinence from self-administration caused their downregulation in NAc shell and STR (Neumaier et al. 2009).

Similar changes in expression patterns have been observed for 5-HT_{2A} receptors after amphetamine administration; namely, McMillen et al. (1991) observed downregulation of 5-HT₂ receptor mRNA in FCx after 7-day experimenter-administered amphetamine treatment. More recent studies examined the effect of escalating dose regimen of passively delivered amphetamine on 5-HT_{2A} receptor mRNA and reported the same direction of changes after 24 h from last amphetamine dose in the cortical areas including PFCx (Horner et al. 2011; Murray et al. 2014). Interestingly, at least in the latter structure, these changes seemed to be transient, since after 4 days of withdrawal 5-HT_{2A} mRNA was upregulated (Murray et al. 2014). Direction of changes may also be structure limited since 5-HT_{2A} mRNA was increased in NAc, STR, and HIP a day after the last drug injection (Horner et al. 2011). Taken together with the current findings, it appears likely that amphetamine transiently induces site specific changes to the expression of multiple 5-HT receptor subtypes. It is possible that those observed changes in 5-HT receptor expression is a system's way of restoring the homeostasis; however, more studies need to be performed to better understand the phenomenon.

Another aspect of this paper was to examine differences between active and passive amphetamine administration. The most important finding from this study was that 20 days of chronic amphetamine exposure triggered elevation of 5-HT_{1B} receptors exclusively in animals that voluntarily administer the drug in the brain regions that are part of reward circuit and are involved in addiction-related behavior. In fact, the mesolimbic dopamine system that projects from VTA to the NAc was numerous reported to be essential for rewarding effects of drugs of abuse (Fibiger and Phillips 1986; Wise and Bozarth 1987; Koob 1992; Wise 1996; McBride et al. 1999; Pierce and Kumaresan 2006). Furthermore, the DA release into the core compartment of NAc is a necessary factor to initiate reward-related instrumental responses (Cardinal et al. 2002) as well as reward-associated learning (Scofield et al. 2016). On the other hand, one of the main outputs of NAc is ventral GP (Heimer and Wilson 1975), a brain region acting as hub for direct and indirect basal ganglia pathway converting limbic motivation signal into motor outputs (Mogenson et al. 1980; Mogenson and Yang 1991) and therefore controlling variety of behaviors, among others, goal-directed actions, decision making, and motivation (Gerfen et al. 1982; Albin et al. 1989; Deniau and Chevalier 1992; Smith et al. 2009; Gerfen and Surmeier 2011; Arimura et al. 2013; Calabresi et al.

2014). Finally, a structure that is also closely connected to the reward circuit is the HIP. It receives a vast dopaminergic input from VTA which has strong influence on learning and memory (Frey et al. 1990, 1991; Matthies et al. 1997; Lisman and Grace 2005; Granado et al. 2008) and also connects back with this structure through indirect loop via NAc and ventral GP (Legault and Wise 1999; Floresco et al. 2003; Lodge and Grace 2006). This way, HIP is well positioned to mediate between reward and limbic areas and to allow the initiation of the further addiction states: compulsive behavior and habit formation (Gerdeman et al. 2003; Everitt et al. 2008; Everitt and Robbins 2013; Everitt 2014). To the best of our knowledge, there is little to none known about the relationship between the 5-HT_{1B} receptors located in the NAc core, GP ventral, and HIP and amphetamine self-administration and goal-directed behaviors. Therefore, we find the result obtained from this study, indicating that 5-HT_{1B} receptors' function(s), at least in above mentioned structures, is directly linked to cognitive processes of addiction and most likely plays a role in incentive drug taking, exciting, and important to the addiction field.

One way to explain this phenomenon could be that only active group was able to learn to anticipate the drug injection and/or its effects whereas others had diminished or abolished expectancy of the substance intake. Even though the passive drug administration has been proven to evoke rewarding effects (i.e., conditioning place preference), the absence of expectancy can make a difference, perhaps increasing the stressful responses instead. As the matter of fact, Dworkin et al. (1995) shown that passive cocaine injections cause higher mortality rate than willful drug self-administration in rats. At the same time, other possible explanation is the differential involvement of DA neurotransmission in animals that undergo active versus passive drug administration; however, data are unequivocal. For instance, one study demonstrated that extracellular levels of DA in the NAc were higher in rats that actively administered cocaine than in those that received the drug passively (Hemby et al. 1997). On the contrary, during amphetamine self-administration, short-term extinction and reinstatement, yoked (but not active) animals exhibited higher DA levels in NAc (Ranaldi et al. 1999). Also experimenter-administered heroin, but not active heroine administration, increased DA release in the latter structure (Hemby et al. 1995). Whether the observed alternation in 5-HT_{1B} receptor expression between active and passive amphetamine administration are evoked by the stress responses, differential DA involvement or not yet named contributors need to be determined; however, their link to incentive drug taking remains rather certain.

Parallel changes in 5-HT_{1B} receptor expression were reported in the remaining groups of animals, which is not unexpected. In the group of animals that were exposed to only single amphetamine session, even though only active rats

were able to titrate the amount and time of the drug delivery, this was the first time exposure to the drug for both, and observed changes might have been due to pharmacological result of amphetamine intake. As for the rodents that underwent amphetamine self-administration followed by 14-day extinction, it is likely that results are caused by lack of drug in the system and perhaps due to the effect of withdrawal-related stress or incubation of amphetamine craving (see above).

Both passive and active administration of amphetamine exerts powerful effect on numerous behavioral functions such as locomotor activity, aggression, mood, and reward circuits (Seiden et al. 1993) in which 5-HT_{1B} receptor engagement was also indicated (Sari 2004). Furthermore, direct relationship between these receptors and amphetamine-induced rewarding behaviors was also demonstrated. Precisely, in amphetamine self-administration model in rats, pharmacological stimulation of 5-HT_{1B} receptor decreased the number of lever presses in fixed (Miszkiewicz et al. 2012) and progressive ratio schedule (Fletcher et al. 2002). Interestingly, Miszkiewicz et al. (2012) concluded that observed reduction in lever presses and drug intake was due to behavioral disruption and not to increase of reward activity. Here, we showed that prolonged amphetamine exposure elevated 5-HT_{1B} receptor expression in numerous structures, including NAc core, which is part of a reward circuit. Therefore, it is possible that agonist-induced behavior observed by Miszkiewicz and others (Miszkiewicz et al. 2012) was caused by ceiling effect. Additionally, a motivational aspect of drug intake may be important. Previous data reported that the same agonist of 5-HT_{1B} receptors enhanced the locomotor hyperactivity induced by amphetamine in mice (Przegaliński et al. 2001), and 5-HT_{1B} receptor overexpression facilitated cocaine-induced locomotor activity in rats (Neumaier et al. 2002). However, it should be noted that in these studies, drugs were passively injected; and in the current study, the increase in 5-HT receptor protein was observed only in animals that administered the drug actively. The reason why active and passive amphetamine administration would lead to opposite behavioral effects is still yet to be determined. On the other hand, 2 weeks of amphetamine extinction led to overexpression of 5-HT_{1B} receptors regardless of animals' drug history. As reported earlier (Miszkiewicz and Przegaliński 2013), pharmacological blockade of these receptors attenuated the amphetamine-evoked reinstatement. Therefore, taken together, it is plausible that by normalizing the 5-HT_{1B} activity, homeostasis would be restored, and drug seeking behavior could be diminished. However, it needs to be mentioned that even though 5-HT_{1B} receptors were elevated during the amphetamine self-administration, the same antagonist remained ineffective in decreasing the amphetamine intake or lever presses during the maintenance phase (Miszkiewicz et al. 2012).

Concluding, this is the first study to demonstrate that single amphetamine session, chronic amphetamine self-administration, and 14-day extinction from this drug alter the expression

of 5-HT_{1B} receptors in various brain regions. Furthermore, increased expression of 5-HT_{1B} receptors after chronic drug intake was limited to the active group of animals, potentially linking 5-HT_{1B} receptor with motivation aspect of addiction. However, several questions regarding the expression pattern of these receptors remain unanswered and need further examination.

Acknowledgments We thank Dr. Malgorzata Frankowska (Department of Drug Addiction Pharmacology, Institute of Pharmacology, Polish Academy of Sciences) for her help performing the experiments and thoughtful discussions and comments on the manuscript.

Author Contribution JM designed the experiments, performed the behavioral and immunochemical experiments, conducted the statistical analyses, created the figures, interpret the results, and wrote the manuscript. JJ performed the behavioral experiments, conducted the statistical analyses, created the figures, and edited the manuscript. EP and MF conceptualized the experiments, oversaw the experimental design, and interpreted the results. EP edited the manuscript.

Funding This research was supported by the grant no. 0034/IP1/2013/72 from the Ministry of Science and Higher Education and the statutory funds of the Institute of Pharmacology.

Compliance with Ethical Standards

The experimental procedures were carried out in accordance with the European Directive 2010/63/EU and were approved by the Bioethical Committee at the Institute of Pharmacology, Polish Academy of Sciences, Krakow.

Conflict of Interest The authors declare that they have no conflict of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Adell A, Celada P, Artigas F (2001) The role of 5-HT_{1B} receptors in the regulation of serotonin cell firing and release in the rat brain. *J Neurochem* 79(1):172–182
- Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. *Trends Neurosci* 12:366–375
- Arimura N, Nakayama Y, Yamagata T, Tanji J, Hoshi E (2013) Involvement of the globus pallidus in behavioral goal determination and action specification. *J Neurosci* 33(34):13639–13653
- Barr JL, Renner KJ, Forster GL (2010) Withdrawal from chronic amphetamine produces persistent anxiety-like behavior but temporally-limited reductions in monoamines and neurogenesis in the adult rat dentate gyrus. *Neuropharmacology* 59:395–405
- Bonhomme N, Cador M, Stinus L, Le Moal M, Spampinato U (1995) Short and long-term changes in dopamine and serotonin receptor

- binding sites in amphetamine-sensitized rats: a quantitative autoradiographic study. *Brain Res* 675(1–2):215–223
- Bronsert MR, Mead AN, Hen R, Rocha BA (2001) Amphetamine-induced locomotor activation in 5-HT(1B) knockout mice: effects of injection route on acute and sensitized responses. *Behav Pharmacol* 12(6–7):549–555
- Bruinvels AT, Palacios JM, Hoyer D (1993) Autoradiographic characterization and localisation of 5-HT1D compared to 5-HT1B binding sites in rat brain. *Naunyn Schmiedeberg's Arch Pharmacol* 347:569–582
- Calabresi P, Picconi B, Tozzi A, Ghiglieri V, Di Filippo M (2014) Direct and indirect pathways of basal ganglia: a critical reappraisal. *Nat Neurosci* 17:1022–1030
- Cardinal RN, Parkinson JA, Lachenal G, Halkerston KM, Rudarakanchana N, Hall J, Morrison CH, Howes SR, Robbins TW, Everitt BJ (2002) Effects of selective excitotoxic lesions of the nucleus accumbens core, anterior cingulate cortex, and central nucleus of the amygdala on autoshaping performance in rats. *Behav Neurosci* 116:553–567
- Compan V, Segu L, Buhot MC, Daszuta A (1998) Selective increases in serotonin 5-HT1B/1D and 5-HT2A/2C binding sites in adult rat basal ganglia following lesions of serotonergic neurons. *Brain Res* 793(1–2):103–111
- Deniau JM, Chevalier G (1992) The lamellar organization of the rat substantia nigra pars reticulata: distribution of projection neurons. *Neuroscience* 46:361–377
- Dworkin SI, Mirkis S, Smith JE (1995) Response-dependent versus response-independent presentation of cocaine: differences in the lethal effects of the drug. *Psychopharmacology* 117:262–266
- Everitt BJ (2014) Neural and psychological mechanisms underlying compulsive drug seeking habits and drug memories—indications for novel treatments of addiction. *Eur J Neurosci* 40:2163–2182
- Everitt BJ, Robbins TW (2013) From the ventral to the dorsal striatum: devolving views of their roles in drug addiction. *Neurosci Biobehav Rev* 37:1946–1954
- Everitt BJ, Belin D, Economidou D, Pelloux Y, Dalley JW, Robbins TW (2008) Review: neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. *Philos Trans R Soc Lond B Biol Sci* 363:3125–3135
- Fabre V, Beaufour C, Evrard A, Rioux A, Hanoun N, Lesch KP, Murphy DL, Lanfumey L, Hamon M, Martres MP (2000) Altered expression and functions of serotonin 5-HT1A and 5-HT1B receptors in knock-out mice lacking the 5-HT transporter. *Eur J Neurosci* 12(7):2299–2310
- Faraone SV (2018) The pharmacology of amphetamine and methylphenidate: relevance to the neurobiology of attention-deficit/hyperactivity disorder and other psychiatric comorbidities. *Neurosci Biobehav Rev* 87:255–270
- Fibiger HC, Phillips AG (1986) Reward, motivation, cognition: psychobiology of mesotelencephalic dopamine systems, *Handbook of physiology. The nervous system*. American Physiological Society, Bethesda
- Filip M, Bader M (2009) Overview on 5-HT receptors and their role in physiology and pathology of the central nervous system. *Pharmacol Rep* 61(5):761–777
- Filip M, Papla I, Nowak E, Jungersmith K, Przegaliński E (2002) Effects of serotonin (5-HT)1B receptor ligands, microinjected into accumbens subregions, on cocaine discrimination in rats. *Naunyn Schmiedeberg's Arch Pharmacol* 366:226–234
- Filip M, Frankowska M, Zaniewska M, Golda A, Przegaliński E (2005) The serotonergic system and its role in cocaine addiction. *Pharmacol Rep* 57:685–700
- Fletcher PJ, Korth KM (1999) RU-24969 disrupts d-amphetamine self-administration and responding for conditioned reward via stimulation of 5-HT1B receptors. *Behav Pharmacol* 10:183–193
- Fletcher PJ, Azampanah A, Korth KM (2002) Activation of 5-HT1B receptors in the nucleus accumbens reduces self-administration of amphetamine on a progressive ratio schedule. *Pharmacol Biochem Behav* 71:717–725
- Floresco SB, West AR, Ash B, Moore H, Grace AA (2003) Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nat Neurosci* 6:968–973
- Frey U, Schroeder H, Matthies H (1990) Dopaminergic antagonists prevent long-term maintenance of posttetanic LTP in the CA1 region of rat hippocampal slices. *Brain Res* 522:69–75
- Frey U, Matthies H, Reymann KG, Matthies H (1991) The effect of dopaminergic D1 receptor blockade during tetanization on the expression of long-term potentiation in the rat CA1 region in vitro. *Neurosci Lett* 129:111–114
- Gerdeman GL, Partridge JG, Lupica CR, Lovinger DM (2003) It could be habit forming: drugs of abuse and striatal synaptic plasticity. *Trends Neurosci* 26:184–192
- Gerfen CR, Surmeier DJ (2011) Modulation of striatal projection systems by dopamine. *Annu Rev Neurosci* 34:441–446
- Gerfen CR, Staines WA, Arbutnot GW, Fibiger HC (1982) Crossed connections of the substantia nigra in the rat. *J Comp Neurol* 207:283–303
- Granado N, Ortiz O, Suarez LM, Martin ED, Cena V, Solis JM, Moratalla R (2008) D1 but not D5 dopamine receptors are critical for LTP, spatial learning, and LTP-induced arc and zif268 expression in the hippocampus. *Cereb Cortex* 18:1–12
- Grimm JW, Hope BT, Wise RA, Shaham Y (2001) Incubation of cocaine craving after withdrawal. *Nature* 412:141–142
- Hajheidari S, Miladi-Gorji H, Bigdeli I (2015) Effects of environmental enrichment during induction of methamphetamine dependence on the behavioral withdrawal symptoms in rats. *Neurosci Lett* 605:39–43
- Heimer L, Wilson RD (1975) The subcortical projections of allocortex: similarities in the neural associations of the hippocampus, the periform cortex and the neocortex. *Golgi centennial symposium proceedings*. Raven Press, New York
- Hemby SE, Martin TJ, Co C, Dworkin SI, Smith JE (1995) The effects of intravenous heroin administration on extracellular nucleus accumbens dopamine concentrations as determined by in vivo microdialysis. *J Pharmacol Exp Ther* 273:591–598
- Hemby SE, Co C, Koves TR, Smith JE, Dworkin SI (1997) Differences in extracellular dopamine concentrations in the nucleus accumbens during response-dependent and response-independent cocaine administration in the rat. *Psychopharmacology* 133(1):7–16
- Hoplight BJ, Vincow ES, Neumaier JF (2007) Cocaine increases 5-HT1B mRNA in rat nucleus accumbens shell neurons. *Neuropharmacology* 52(2):444–449
- Horner KA, Gilbert YE, Noble ES (2011) Differential regulation of 5-HT2A receptor mRNA expression following withdrawal from a chronic escalating dose regimen of D-amphetamine. *Brain Res* 1390:10–20
- Hoyer D, Hannon JP, Martin GR (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol Biochem Behav* 71:533–554
- Kindlundh-Högberg AM, Svenningsson P, Schiöth HB (2006) Quantitative mapping shows that serotonin rather than dopamine receptor mRNA expressions are affected after repeated intermittent administration of MDMA in rat brain. *Neuropharmacology* 51(4):838–847
- Koob GF (1992) Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol Sci* 13:177–184
- Kuczenski R, Segal DS (1997) Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: comparison with amphetamine. *J Neurochem* 68(5):2032–2037
- Legault M, Wise RA (1999) Injections of N-methyl-D-aspartate into the ventral hippocampus increase extracellular dopamine in the ventral tegmental area and nucleus accumbens. *Synapse* 31:241–249
- Li H, Scholl JL, Tu W, Hassell JE, Watt MJ, Forster GL, Renner KJ (2014) Serotonergic responses to stress are enhanced in the central

- amygdala and inhibited in the ventral hippocampus during amphetamine withdrawal. *Eur J Neurosci* 40(11):3684–3692
- Li X, Zeric T, Kambhampati S, Bossert JM, Shaham Y (2015) The central amygdala nucleus is critical for incubation of methamphetamine craving. *Neuropsychopharmacology* 40:1297–1306
- Lisman JE, Grace AA (2005) The hippocampal-VTA loop: controlling the entry of information into long-term memory. *Neuron* 46:703–713
- Lodge DJ, Grace AA (2006) The hippocampus modulates dopamine neuron responsiveness by regulating the intensity of phasic neuron activation. *Neuropsychopharmacology* 31:1356–1361
- Manrique C, François-Bellan AM, Segu L, Becquet D, Héry M, Faudon M, Héry F (1994) Impairment of serotonergic transmission is followed by adaptive changes in 5HT1B binding sites in the rat suprachiasmatic nucleus. *Brain Res* 663(1):93–100
- Matthies H, Becker A, Schroeder H, Kraus J, Hollt V, Krug M (1997) Dopamine D1-deficient mutant mice do not express the late phase of hippocampal long-term potentiation. *NeuroReport* 8:3533–3535
- McBride WJ, Murphy JM, Ikemoto S (1999) Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. *Behav Brain Res* 101:129–152
- McMillen BA, Scott SM, Williams HL (1991) Effects of subchronic amphetamine or amfonelic acid on rat brain dopaminergic and serotonergic function. *J Neural Transm Gen Sect* 83(1–2):55–66
- Mead AN, Zamanillo D, Becker N, Stephens DN (2007) AMPA receptor GluR1 subunits are involved in the control over behavior by cocaine-paired cues. *Neuropsychopharmacology* 32:343–353
- Miszkiel J, Przegaliński E (2013) Effects of serotonin (5-HT)1B receptor ligands on amphetamine-seeking behavior in rats. *Pharmacol Rep* 65(4):813–822
- Miszkiel J, Adamczyk P, Filip M, Przegaliński E (2012) The effect of serotonin 5HT1B receptor ligands on amphetamine self-administration in rats. *Eur J Pharmacol* 677(1–3):111–115
- Mogenson GJ, Yang CR (1991) The contribution of basal forebrain to limbic-motor integration and the mediation of motivation to action. *Adv Exp Med Biol* 295:267–290
- Mogenson GJ, Jones DL, Yim CY (1980) From motivation to action: functional interface between the limbic system and the motor system. *Prog Neurobiol* 14:69–97
- Murray RC, Hebbard JC, Logan AS, Vanchipurakel GA, Gilbert YE, Homer KA (2014) Stress and withdrawal from d-amphetamine alter 5-HT2A receptor mRNA expression in the prefrontal cortex. *Neurosci Lett* 559:44–49
- Neisewander JL, Baker DA, Fuchs RA, Tran-Nguyen LT, Palmer A, Marshall JF (2000) Fos protein expression and cocaine-seeking behavior in rats after exposure to a cocaine self-administration environment. *J Neurosci* 20:798–805
- Neumaier JF, Vincow ES, Arvanitogiannis A, Wise RA, Carlezon WA (2002) Elevated expression of 5-HT1B receptors in nucleus accumbens efferents sensitizes animals to cocaine. *J Neurosci* 22:10856–10863
- Neumaier JF, McDevitt RA, Polis IY, Parsons LH (2009) Acquisition of and withdrawal from cocaine self-administration regulates 5-HT mRNA expression in rat striatum. *J Neurochem* 111(1):217–227
- O'Dell LE, Manzardo AM, Polis I, Stouffer DG, Parsons LH (2006) Biphasic alterations in serotonin-1B (5-HT1B) receptor function during abstinence from extended cocaine self-administration. *J Neurochem* 99:1363–1376
- Papla I, Filip M, Przegaliński E (2002) Effect of intra-tegmental micro-injections of 5-HT1B receptor ligands on the amphetamine-induced locomotor hyperactivity in rats. *Pol J Pharmacol* 54:351–357
- Parada M, Hernandez L, Schwartz D, Hoebel BG (1988) Hypothalamic infusion of amphetamine increases serotonin, dopamine and norepinephrine. *Physiol Behav* 44(4–5):607–610
- Parent A, Descarries L, Beaudet A (1981) Organization of ascending serotonin systems in the adult rat brain. A radioautographic study after intraventricular administration of [³H]5-hydroxytryptamine. *Neuroscience* 6(2):115–138
- Parsons LH, Weiss F, Koob GF (1998) Serotonin1B receptor stimulation enhances cocaine reinforcement. *J Neurosci* 18:10078–10089
- Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates. American Press Inc., San Diego
- Pazos A, Palacios JM (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. *Brain Res* 346:205–230
- Pentkowski NS, Acosta JI, Browning JR, Hamilton EC, Neisewander JL (2009) Stimulation of 5-HT(1B) receptors enhances cocaine reinforcement yet reduces cocaine-seeking behavior. *Addict Biol* 14(4):419–430
- Pentkowski NS, Cheung TH, Toy WA, Adams MD, Neumaier JF, Neisewander JL (2012) Protracted withdrawal from cocaine self-administration flips the switch on 5-HT(1B) receptor modulation of cocaine abuse-related behaviors. *Biol Psychiatry* 72(5):396–404
- Pentkowski NS, Harder BG, Brunwasser SJ, Bastle RM, Peartree NA, Yanamandra K, Adams MD, Der-Ghazarian T, Neisewander JL (2014) Pharmacological evidence for an abstinence-induced switch in 5-HT1B receptor modulation of cocaine self-administration and cocaine-seeking behavior. *ACS Chem Neurosci* 5(3):168–176
- Pickens CL, Airavaara M, Theberge F, Fanous S, Hope BT, Shaham Y (2011) Neurobiology of the incubation of drug craving. *Trends Neurosci* 34:411–420
- Pierce RC, Kumaresan V (2006) The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? *Neurosci Biobehav Rev* 30:215–238
- Przegaliński E, Siwanowicz J, Nowak E, Papla I, Filip M (2001) Role of 5-HT1B receptors in the sensitization to amphetamine in mice. *Eur J Pharmacol* 422:91–99
- Przegaliński E, Czepiel K, Nowak E, Dlaboga D, Filip M (2003) Withdrawal from chronic cocaine up-regulates 5-HT1B receptors in the rat brain. *Neurosci Lett* 351(3):169–172
- Przegaliński E, Golda A, Frankowska M, Zaniewska M, Filip M (2007) Effects of serotonin 5-HT1B receptor ligands on the cocaine- and food-maintained self-administration in rats. *Eur J Pharmacol* 559:165–172
- Przegaliński E, Golda A, Filip M (2008) Effects of serotonin (5-HT)(1B) receptor ligands on cocaine-seeking behavior in rats. *Pharmacol Rep* 60:798–810
- Pum M, Carey RJ, Huston JP, Müller CP (2007) Dissociating effects of cocaine and d-amphetamine on dopamine and serotonin in the perirhinal, entorhinal, and prefrontal cortex of freely moving rats. *Psychopharmacology* 193(3):375–390
- Ranaldi R, Pocock D, Zerek R, Wise RA (1999) Dopamine fluctuations in the nucleus accumbens during maintenance, extinction, and reinstatement of intravenous D-amphetamine self-administration. *J Neurosci* 19:4102–4109
- Reinbold ED, Scholl JL, Oliver KM, Watt MJ, Forster GL (2014) Central CRF2 receptor antagonism reduces anxiety states during amphetamine withdrawal. *Neurosci Res* 89:37–43
- Salomon L, Lanteri C, Glowinski J, Tassin JP (2006) Behavioral sensitization to amphetamine results from an uncoupling between noradrenergic and serotonergic neurons. *Proc Natl Acad Sci U S A* 103(19):7476–7481
- Sari Y (2004) Serotonin1B receptors: from protein to physiological function and behavior. *Neurosci Biobehav Rev* 28:565–582
- Sari Y, Lefèvre K, Bancila M, Quignon M, Miquel MC, Langlois X, Hamon M, Vergé D (1997) Light and electron microscopic immunocytochemical visualization of 5-HT1B receptors in the rat brain. *Brain Res* 760:281–286
- Sari Y, Miquel MC, Brisorgueil MJ, Ruiz G, Doucet E, Hamon M, Vergé D (1999) Cellular and subcellular localization of 5-hydroxytryptamine1B receptors in the rat central nervous system: immunocytochemical, autoradiographic and lesion studies. *Neuroscience* 88:899–915

- Scofield MD, Heinsbroek JA, Gipson CD, Kupchik YM, Spencer S, Smith AC, Roberts-Wolfe D, Kalivas PW (2016) The nucleus accumbens: mechanisms of addiction across drug classes reflect the importance of glutamate homeostasis. *Pharmacol Rev* 68(3):816–871
- Seiden LS, Sabol KE, Ricaurte GA (1993) Amphetamine: effects on catecholamine systems and behavior. *Annu Rev Pharmacol Toxicol* 33(1):639–677
- Sexton TJ, McEvoy C, Neumaier JF (1999) (+) 3,4-methylenedioxymethamphetamine ('ecstasy') transiently increases striatal 5-HT_{1B} binding sites without altering 5-HT_{1B} mRNA in rat brain. *Mol Psychiatry* 4(6):572–579
- Smith KS, Tindell AJ, Aldridge JW, Berridge KC (2009) Ventral pallidum roles in reward and motivation. *Behav Brain Res* 196(2):155–167
- Steinbusch HW (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience* 6(4):557–618
- Swinford-Jackson SE, Anastasio NC, Fox RG, Stutz SJ, Cunningham KA (2016) Incubation of cocaine cue reactivity associates with neuroadaptations in the cortical serotonin (5-HT) 5-HT_{2C} receptor (5-HT_{2CR}) system. *Neuroscience* 324:50–61
- Tu W, Cook A, Scholl JL, Mears M, Watt MJ, Renner KJ, Forster GL (2014) Serotonin in the ventral hippocampus modulates anxiety-like behavior during amphetamine withdrawal. *Neuroscience* 281:35–43
- Van Oekelen D, Luyten WH, Leysen JE (2003) 5-HT_{2A} and 5-HT_{2C} receptors and their atypical regulation properties. *Life Sci* 72(22):2429–2449
- Venniro M, Zhang M, Shaham Y, Caprioli D (2017) Incubation of methamphetamine but not heroin craving after voluntary abstinence in male and female rats. *Neuropsychopharmacology* 42(5):1126–1135
- Vuong SM, Oliver HA, Scholl JL, Oliver KM, Forster GL (2010) Increased anxiety-like behavior of rats during amphetamine withdrawal is reversed by CRF₂ receptor antagonism. *Behav Brain Res* 208(1):278–281
- Wise RA (1996) Neurobiology of addiction. *Curr Opin Neurobiol* 6:243–251
- Wise RA, Bozarth MA (1987) A psychomotor stimulant theory of addiction. *Psychol Rev* 94:469–492