Published in final edited form as:

Eur J Pharmacol. 2017 February 05; 796: 207–214. doi:10.1016/j.ejphar.2017.01.001.

Blockade of patch-based μ opioid receptors in the striatum attenuates methamphetamine-induced conditioned place preference and reduces activation of the patch compartment

Kristen A. Horner, Mary Caroline Logan, Trevor J. Fisher, and Jordan B. Logue

Division of Basic Medical Sciences, Mercer University School of Medicine, Macon, GA 31207

Abstract

The behavioral effects of methamphetamine (METH) are mediated by the striatum, which is divided into the patch compartment, which mediates limbic and reward functions, and the matrix compartment, which mediates sensorimotor tasks. METH treatment results in repetitive behavior that is related to enhanced relative activation of the patch versus the matrix compartment. The patch, but not the matrix compartment contains a high density of μ opioid receptors, and localized blockade of patch-based μ opioid receptors attenuates METH-induced patch-enhanced activity and repetitive behaviors. Numerous studies have examined patch-enhanced activity and the contribution of patch-associated μ opioid receptors to METH-induced repetitive behavior, but it is not known whether patch-enhanced activity occurs during METH-mediated reward, nor is it known if patch-based μ opioid receptors contribute to METH reward. The goals of this study were to determine if blockade of patch-based μ opioid receptors alters METH-induced conditioned place preference (CPP), as well activation of the patch and matrix compartments following METH-mediated CPP. A biased conditioning paradigm was used to assess CPP, and conditioning occurred over an 8-day period. Animals were bilaterally infused in the striatum with the μ -specific antagonist CTAP or vehicle prior to conditioning. Animals were tested for preference 24h after the last day of conditioning, sacrificed and the brains processed for immunohistochemistry. Blockade of patch-based μ opioid receptors reduced METH-induced CPP, and reduced patch-enhanced c-Fos expression in the striatum following METH-mediated CPP. These data indicate that patch-enhanced activity is associated with METH-mediated reward and patch-based μ opioid receptors contribute to this phenomenon.

Keywords

psychostimulant; basal ganglia; immediate early gene; reward; behavior

Corresponding Author: Kristen A. Horner, Ph.D., 1550 College St., Macon, GA 31207, 478-301-4050 (office), 478-301-5489 (fax), horner_ka@mercer.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Statement of Conflict of Interest

The authors declare that there is no conflict interest.

1. Introduction

The striatum mediates the behavioral effects of psychostimulants, and can be divided into the patch (striosome) and matrix compartments based on differences in neurochemistry and neuroanatomy. The spiny neurons of the patch compartment express a high density of μ opioid receptors, while the spiny neurons of the matrix compartment express few μ opioid receptors, but are enriched in calbindin (Gerfen et al., 1985; Herkenham and Pert, 1981; Pert et al., 1976). The patch compartment receives input from limbic cortices, and projects almost exclusively to the substantia nigra pars compacta (SNpc), which sends dopaminergic inputs back to the striatum (Fujiyama et al., 2011; Gerfen, 1984; Tokuno et al., 2002), while the matrix compartment receives input from sensorimotor cortices and comprises the majority of the direct and indirect pathways that mediate movement (Crittenden and Graybiel, 2011; Fujiyama et al., 2011; Gerfen, 1984; Gerfen, 1989). Based on these differences, the activity of the patch and matrix compartments are likely regulated by diverse mechanisms, and mediate different aspects of behavior.

While the matrix compartment is related to sensorimotor processing, the limbic-based circuits of the patch compartment mediate repetitive behaviors such as psychostimulant-induced stereotypy (Canales, 2005; Canales and Graybiel, 2000; Crittenden and Graybiel, 2011). Enhanced relative activation of the patch versus matrix compartment is observed during psychostimulant-induced stereotypy, and the degree of stereotypy is positively correlated with patch-enhanced activation in dorsolateral striatum (Canales and Graybiel, 2000). Since the patch compartment has dense connectivity with regions that process emotional information, stereotypy is likely due to the enhanced relative activation of patch-based circuits, resulting in behaviors that are driven by internal emotional states, at the expense of switching to adaptive behaviors based on the external environment (Aliane et al., 2009; Canales, 2005; Canales and Graybiel, 2000; Crittenden and Graybiel, 2011). The relative enhanced activation of the patch compartment could contribute to the development of habitual drug abuse, due to the interactions of the patch compartment with regions that mediate reinforcement and relapse into drug-seeking behavior (Canales, 2005). Of note, previous work has shown that animals will repeatedly electrically self-stimulate when electrodes are placed in or near the patch compartment, supporting the notion that this region plays a role in processes that guide motivation and reward learning (White and Hiroi, 1998). However, whether enhanced relative activation of the patch versus matrix compartment is associated with psychostimulant-mediated reward is unknown, and the mechanisms that contribute to patch-enhanced activity during psychostimulant-mediated reward not identified.

Striatal μ opioid receptors, since they are expressed specifically on the neurons of the patch compartment are in an optimal position to modulate the activity of this region. Our previous work indicated that blockade of striatal μ opioid receptors attenuated patch-enhanced activation and stereotypy following METH treatment (Horner et al., 2010). While it has been shown that central μ opioid receptors modulate psychostimulant-mediated reward (Schroeder et al., 2007), the specific contribution of patch-based μ opioid receptors is unknown. These data indicate that μ opioid receptors play a role in the activation of neurons of the patch compartment following psychostimulant treatment, as well as psychostimulant-induced

behaviors. However, whether patch-based μ opioid receptors contribute to patch-enhanced activation in the context of METH-mediated reward has not been examined. The goals of this study were to determine if patch-enhanced activity is associated with METH-mediated reward, and whether patch-based μ opioid receptors contribute to this phenomenon.

2. Materials and Methods

2.1 Drugs

($\pm$)METH-HCl was generously provided by the National Institute on Drug Abuse, and injected subcutaneously in a volume of 1 ml/kg body weight. The dose of METH (2 mg/kg; (Suzuki and Misawa, 1995) was based on the weight of the salt. The μ opioid receptor antagonist CTAP (D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr) was obtained from Tocris Bioscience (Minneapolis, MN, USA) and dissolved in sterile buffered artificial cerebrospinal fluid (aCSF; 144 mM NaCl; 2.68 mM KCl; 1.6 mM CaCl_2 ; 2.6 mM MgCl_2 ; 0.4 mM KH_2PO_4 , pH, 7.2) at 0.8 $\mu\text{g}/\mu\text{l}$ (Schroeder et al., 2007).

2.2. Animals and surgery

Male Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC, USA), weighing 250–350g were used in all experiments. Rats were housed in plastic cages in a temperature-controlled room on a 14:10 h light/dark cycle with free access to food and water. All animal care and experimental manipulations were approved by the Institutional Animal Care and Use Committee of Mercer University School of Medicine and were in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. The minimum possible number of animals (based on power analyses) was used for our experiments and measures were taken to minimize any suffering that might occur during our procedures.

One week after arrival at our facility, rats underwent surgery for the placement of bilateral indwelling intrastriatal cannulae. Rats were anesthetized with ketamine (90 mg/kg, i.p.) and xylazine (9 mg/kg, i.p.) and stainless steel 26-gauge guide cannulae 3.5 mm in length (Plastics One, Roanoke, VA, USA) were implanted bilaterally, based on the coordinates of Paxinos and Watson (Paxinos and Watson, 2005) in the rostral striatum, at +1.5 mm from bregma, ± 3.0 mm lateral to midline and 3.5 mm below the surface of the skull. The cannulae were secured to the skull with dental acrylic and anchored with stainless steel surgical screws inserted into the skull. The guide cannulae were kept patent with 31-gauge obturators that were the same length as the guides. Rats were allowed to recover for 2–3 days before the start of the experiment. Only rats whose cannulae were in the rostral striatum were included in subsequent analyses.

2.3. Conditioned Place Preference (CPP)

Conditioning chambers consisted of a 30 $\times$ 30 $\times$ 30-cm Plexiglass chamber divided into two distinct compartments separated by a removable partition. One compartment had solid gray walls, while the other compartment had vertical alternating black and white stripes that were 2.5 cm wide. Floors were stainless steel wire mesh of differing mesh size to provide differential tactile cues. One day before the start of the conditioning procedure, rats were

habituated chambers for 30 min with the partition removed. Twenty-four h later, the rats were returned to the chambers for 20 min with the partition removed, and the time on each side recorded to determine each animal's initial bias. Conditioning began the next day, and consisted of 8 days of alternating saline or METH injections, with rats confined to the preferred side, as determined by the preconditioning bias test, on saline exposure days (days 1, 3, 5 and 7 of conditioning), and the initially non-preferred side on drug exposure days (days 2, 4, 6, and 8 of conditioning; (Nygard et al., 2015)). Rats were randomly assigned to one of four groups: vehicle/saline, vehicle/METH, CTAP/saline, or CTAP/METH. Prior to each conditioning session, the dummy cannulae were removed and 31-gauge stainless steel injection cannulae that extended 1.5 mm beyond the guide were inserted into the guide cannulae. A 5- μ l volume of aCSF or CTAP (4 μ g/5 μ l; (Schroeder et al., 2007)) was administered bilaterally at a rate of 1.0 μ l/minute to the freely moving animal. After each infusion, the injection cannulae were left in place for 1 minute in order to minimize fluid back flow through the cannulae. Twenty min later, each rat received an injection of saline or METH, and was immediately confined to the conditioning chamber for 30 min. On drug exposure days (i.e., days 2, 4, 6 and 8 in the initially non-preferred chamber), rats in the vehicle/METH and vehicle/saline groups were intrastratially infused with aCSF, while animals in the CTAP/METH and CTAP/saline groups were intrastratially infused with CTAP prior to their injections (Schroeder et al., 2007). On saline exposure days (i.e., days 1, 3, 5 and 7 in the initially preferred chamber) all groups of rats were infused with aCSF prior to injection with saline (Schroeder et al., 2007). Twenty-four h after the last conditioning session, rats underwent preference testing, with rats in the drug-free state given free access to both chambers for 20 min. The difference in seconds between the time spent in the METH-paired compartment during the preference testing and the time spent in the same initially non-preferred compartment during the bias testing prior to starting the conditioning procedure was used to calculate the degree of place conditioning (Schroeder et al., 2007). For saline-treated rats, preference was calculated as the difference in seconds between the time spent in the initially non-preferred compartment during the preference testing and the time spent in the same non-preferred compartment prior to conditioning, as previously described (Schroeder et al., 2007). A positive preference score indicates a conditioned place preference, while a negative preference score is indicative of a conditioned place aversion (Schroeder et al., 2007). Both the bias test and preference test sessions were digitally recorded and analyzed *post-hoc* using AnyMaze software. In order to determine whether differences in locomotor activity may have contributed to the animals' behavior in the CPP, *post-hoc* analysis of the distance traveled during the preference test was analyzed using AnyMaze software.

2.4. Tissue processing for immunohistochemistry

Thirty min after preference testing, rats were killed by exposure to CO₂ for 1 min followed by decapitation. The brains were rapidly harvested, flash-frozen in isopentane and stored at -80°C until they were cut into 12- μ m sections through the striatum at the level of the infusion (approximately + 1.5 mm anterior to bregma (Paxinos and Watson, 2005) on a cryostat (Minotome Plus, Triangle Biomedical Sciences, Durham, NC, USA).

2.5. c-Fos immunohistochemistry

Sections were post-fixed in 4% paraformaldehyde, pH 7.4 and then rinsed three times in phosphate-buffered saline (PBS). Slides were then blocked with 4% normal goat serum (NGS)/0.3% Triton X-100 (TX) for 1 h followed by overnight incubation at 4°C with a polyclonal antibody for c-Fos (Abcam, Cambridge, MA, USA), diluted in 1:1,000 in 0.3 % TX/0.1 M PBS. The slides were then washed several times in PBS and incubated for 2 h at room temperature in biotinylated goat anti-rabbit IgG antiserum (Vector Laboratories, Burlingame, CA, USA) diluted 1:200 in 0.1 M PBS/1% NGS. Slides were then washed three times in PBS, incubated 1 h in ABC solution (Elite ABC Kit, Vector Laboratories) and washed three more times in PBS. Bound antibody was detected using a 3,3'-diaminobenzidine/Ni⁺ solution (Vector Laboratories). Slides were washed with deionized H₂O, dehydrated in a series of alcohols and coverslipped out of xylene.

2.6. μ opioid receptor immunohistochemistry

Sections that were 12- μ m from the sections labeled for c-Fos were labeled for the μ opioid receptor in order to delineate the patch and matrix compartments. Sections were post-fixed in 4% paraformaldehyde/0.9% NaCl and rinsed three times in 0.1 M PBS. Slides were then blocked with 10% bovine serum albumin (BSA)/0.3% TX/0.1 M PBS for 2 h followed by overnight incubation at 4°C with a polyclonal antibody for the μ opioid receptor (Immunostar, Hudson, WI, USA), diluted in 1:1,000 in 0.3% TX/0.1 M PBS/5% BSA. The slides were then washed several times in PBS and incubated for 2 h at room temperature in biotinylated goat anti-rabbit IgG antiserum (Vector Laboratories) diluted 1:200 in 0.1 M PBS/5% BSA. Slides were then washed three times in PBS, incubated 1 h in ABC solution (Elite ABC Kit, Vector Laboratories) and washed three more times in PBS. Bound antibody was detected using a 3,3'-diaminobenzidine/Ni⁺ solution (Vector Laboratories). Slides were washed with deionized H₂O, dehydrated in a series of alcohols and coverslipped out of xylene.

2.7. Image analysis

c-Fos-labeled sections and the adjacent μ opioid receptor-labeled sections (7–10 animals/treatment group) were captured by a VistaVision microscope (VWR, Radnor, PA, USA) with a video camera (CCD Moticam 2300, Motic, Richmond, BC, Canada) using a 4 $\times$ objective. Image J was used to outline regions of either dense μ opioid receptor immunoreactivity (patch) or absent μ opioid receptor immunoreactivity (matrix), which were superimposed over the corresponding areas of the adjacent c-Fos-labeled striatal section (Murray et al., 2014; Murray et al., 2015). The number c-Fos-labeled particles that exceeded the threshold density in each region of interest was determined using the particle analysis option in Image J. Prior to analysis, the pixel range for particle size was determined by outlining approximately 15–20 positively labeled cells from 10 to 15 randomly selected sections and determining the average size of the labeled cells in terms of pixel area. The lower limit for a “labeled cell” on the particle analysis setting was then set to the smallest number of pixels measured for any cell, whereas the upper limit was set at the maximal particle size on the particle analysis option on Image J. The threshold density was adjusted such that background staining was eliminated and the number of immunoreactive pixels per the

selected area in each region of interest was measured above this threshold. Counts of c-Fos-labeled particles were expressed as the number c-Fos-immunoreactive particles per mm².

2.8. Statistical analysis

The effects of μ opioid receptor blockade on METH-induced CPP, locomotor activity, c-Fos immunoreactivity in the patch and matrix compartments and the ratio of patch-to-matrix c-Fos immunoreactivity (which was calculated by dividing the number of c-Fos immunoreactive particles/mm² in the patch divided by the number of c-Fos immunoreactive particles/mm² in the matrix for each animal) was analyzed using a two-way (pre-treatment x treatment condition) analysis of variance. *Post-hoc* analysis of significant effects was accomplished using individual Bonferroni (Dunn) *t*-tests. The alpha level for all analyses was set at 0.05. In the case of a significant overall main effect of treatment, the alpha level was not corrected, as only one comparison was made in the *post-hoc* analysis (SAL vs. METH). For the *post-hoc* analysis of significant interaction terms, four comparisons were made (VEH/SAL vs. VEH/METH; VEH/SAL vs. CTAP/SAL; VEH/METH vs. CTAP/METH; CTAP/SAL vs. CTAP/METH), thus requiring a *p*-value = 0.0125 (0.05/4) for statistical significance. A one-sample *t*-test against zero was also used to determine whether a significant place preference or aversion was observed following treatment (Schroeder et al., 2007).

3. Results

3.1. The effects of patch-based μ opioid receptor blockade on METH-induced CPP

CPP was used to assess the role of μ opioid receptors expressed on the neurons of the patch compartment in the development of METH reward. A two-way analysis of variance of the preference scores, which were generated by calculating the difference in the amount of time spent in the drug-paired chamber vs. the amount of time spent in the initially preferred chamber (Schroeder et al., 2007), revealed a significant main effect of CTAP pretreatment ($F_{1,29}=5.704$, $P=0.024$), a significant main effect of METH treatment ($F_{1,29}=5.601$, $P=0.025$) and a significant pretreatment x treatment interaction ($F_{1,29}=7.542$; $P=0.01$; Fig. 1). *Post-hoc* analysis revealed that METH treatment induced a significant place preference in VEH-pretreated animals, as compared to VEH-pretreated, saline-treated animals ($P=0.005$; $t=3.31$). Blockade of patch-based μ opioid receptors with CTAP prevented the development METH-induced place preference, as there was a significant difference between CTAP-pretreated, METH-treated animals and VEH-pretreated, METH-treated animals ($P=0.003$; $t=3.72$), but not between CTAP-pretreated, SAL-treated animals and CTAP-pretreated, METH-treated animals ($P=0.764$, $t=0.306$). Intrastratial administration of CTAP in SAL-treated animals did not result in a significant place aversion (one-sample *t*-test against zero: $t=0.88$, $P=0.41$), and intrastratial administration of VEH in SAL-treated animals did not produce a significant place preference or aversion (one-sample *t*-test against zero: $t=0.521$, $P=0.616$). These data indicate that blockade of patch-based μ opioid receptors attenuates METH-mediated reward. There were no significant effects of pretreatment ($F_{1,25}=4.1$; $P=0.06$) or treatment ($F_{1,25}=1.6$; $P=0.21$) on the degree of locomotor activity during the preference test, nor was there a significant pretreatment x treatment interaction ($F_{1,25}=0.54$; $P=0.47$; Table 1).

3.2. The effects of patch-based μ opioid receptor blockade on c-Fos expression in the patch and matrix compartments following METH-induced CPP

The development of METH-induced CPP appeared to increase c-Fos immunoreactivity in the patch compartment relative to the matrix, an effect that was reduced by pre-treatment with CTAP (Fig. 2). Quantitative analysis of c-Fos immunoreactivity in the patch compartment, followed by a two-way analysis of variance revealed significant main effects of CTAP pretreatment ($F_{1,26}=7.07$; $P=0.0132$) and METH treatment ($F_{1,26}=5.57$; $P=0.026$), as well as a significant pretreatment $\times$ treatment interaction ($F_{1,26}=10.79$; $P=0.0029$). *Post-hoc* analysis revealed that there was a significant increase in the number of c-Fos immunoreactive particles/mm² in the patch compartment of VEH-pretreated, METH-treated animals, as compared to VEH-pretreated, saline-treated animals ($P=0.0032$; $t=3.55$). Pretreatment with CTAP significantly reduced the number of c-Fos immunoreactive particles/mm² in the patch compartment of METH-treated animals, as there was a significant difference between VEH/METH and CTAP/METH groups ($P=0.010$; $t=3.12$). There was no significant difference between in the number of c-Fos immunoreactive particles/mm² in the patch compartment for CTAP/METH vs. CTAP/SAL groups ($P=0.461$; $t=0.913$), nor did CTAP treatment alone significantly alter the number of c-Fos immunoreactive particles/mm² in the patch compartment ($P=0.68$; $t=0.422$; Fig. 3).

Quantitative analysis of the number of c-Fos immunoreactive particles in the matrix compartment, followed by a two-way analysis of variance, revealed a significant main effect of METH treatment ($F_{1,33}=6.08$; $P=0.019$), but no significant effect of CTAP pretreatment ($F_{1,33}=0.222$; $P=0.640$) and no significant pretreatment $\times$ interaction ($F_{1,33}=0.23$; $P=0.66$). *Post-hoc* analysis revealed that METH treatment decreased the number of c-Fos immunoreactive particles/mm² in the matrix compartment regardless of the pretreatment that was given ($P=0.021$; $t=2.43$). The data indicate that blockade of patch-based μ opioid receptors reduces the activation of patch compartment following METH-mediated reward (Fig. 3).

3.3. The effects of patch-based μ opioid receptor blockade on the relative activation of the patch vs. matrix compartments following METH-induced CPP

METH-mediated CPP was associated with increased c-Fos immunoreactivity in the patch compartment and a down-regulation of c-Fos immunoreactivity in the matrix compartment, and the relative level of compartmental activation differed as shown by the ratio of patch-to-matrix c-Fos immunoreactivity (Fig. 4). Two-way analysis of variance of the patch-to-matrix ratio of c-Fos immunoreactivity revealed significant main effects of CTAP pretreatment ($F_{1,32}=19.29$; $P=0.0001$) and METH treatment ($F_{1,32}=20.62$; $P<0.0001$), and a significant pretreatment $\times$ treatment interaction ($F_{1,23}=16.25$; $P=0.0003$). *Post-hoc* analysis of significant effects found that there was a significant increase in the ratio of patch-to-matrix c-Fos immunoreactivity in VEH-pretreated, METH-treated animals, as compared to VEH-pretreated, SAL-treated animals ($P=0.0002$; $t=4.78$). Pretreatment with CTAP attenuated the METH-induced ratio of patch-to-matrix c-Fos immunoreactivity, as *post-hoc* analysis also revealed that there was a significant difference in the ratio of patch-to-matrix c-Fos immunoreactivity between VEH-pretreated, METH-treated animals, and CTAP-pretreated, METH treated animals ($P=0.0004$; $t=4.68$). The ratio of patch-to-matrix c-Fos

immunoreactivity was not significantly different between CTAP-pretreated, SAL-treated animals and CTAP-pretreated, METH-treated animals ($P=0.511$; $t=0.673$), nor was there a difference between VEH-pretreated, SAL-treated animals and CTAP-pretreated, SAL-treated animals ($P=0.683$; $t=0.414$). These data indicate that following METH-mediated reward, the relative activation of the patch compartment is enhanced as compared to the matrix compartment, and blockade of patch-based μ opioid receptors can attenuate this effect by reducing c-Fos activation within the patch compartment.

4. Discussion

The goals of this study were to determine if a pattern-enhanced pattern of activation in the striatum is associated with METH-mediated reward and the contribution of patch-based μ opioid receptors. A patch-enhanced pattern of activation was observed in the rostral striatum following METH-induced CPP, as c-Fos levels were increased in the patch compartment and decreased in the matrix compartment in animals that underwent METH-mediated CPP. Intrastratial administration of CTAP attenuated METH-induced CPP and diminished the patch-enhanced pattern of activation associated with METH-induced CPP. These data indicate that METH-mediated reward is associated with preferential enhancement of patch-based circuits, and that activation of patch-based μ opioid receptors during METH exposure contributes to the enhanced relative activity of the patch compartment, and the development of METH-mediated reward association.

Numerous studies have shown that enhanced activation of the patch compartment relative to the matrix compartment is observed during psychostimulant-induced stereotypy, and our studies have shown that an intact patch compartment is necessary for the expression of repetitive behaviors induced by psychostimulants (Adams et al., 2003; Canales and Graybiel, 2000; Graybiel et al., 1990; Horner et al., 2012; Horner and Keefe, 2006; Horner et al., 2010; Jedynek et al., 2012; Murray et al., 2014; Saka et al., 2004; Wang et al., 1995). The current study is the first that demonstrate that drug-mediated reward is also associated with enhanced activation of the patch compartment relative to the matrix compartment. Enhanced relative activation of the patch compartment may play a role in habit learning, including maladaptive behaviors such as addiction, although to date no study has directly examined this hypothesis (Canales, 2005; Canales and Graybiel, 2000). While the nucleus accumbens has received the most attention regarding reward, the compulsive and rigid behaviors that are typical of addiction are in line with the types of compulsive and habitual behaviors that are mediated by basal ganglia circuits through the striatum. A number of studies have shown that the dorsolateral striatum is a key region for the development of habitual behaviors, and pharmacologically induced repetitive and fixed behaviors are associated with patch-enhanced activation specifically in the dorsolateral striatum (Canales and Graybiel, 2000; Yin et al., 2004). It is possible that the initial learning processes involving drug-seeking behaviors may rely on the nucleus accumbens, but as the behavior becomes more rigid and fixed, drug-seeking behaviors may become more reliant on the dorsolateral striatum (Berke and Hyman, 2000; Canales, 2005; Hyman and Malenka, 2001; Natarajan and Yamamoto, 2011; Robbins and Everitt, 2002; Voorn et al., 2004). In support this notion, following extended psychostimulant self-administration the presentation of a conditioned stimulus previously paired with the drug alters dopamine release in the

dorsolateral striatum, but not in the nucleus accumbens (Vanderschuren and Kalivas, 2000). Furthermore, ascending recurrent interconnected loops exist between the nucleus accumbens and the dorsolateral striatum via midbrain dopamine neurons, indicating that an anatomical pathway exists for the transfer of limbic information to regions that mediate habitual learning (Haber et al., 2000). This system could participate in transferring limbic-related information to the patch compartment, resulting in a shift in control of actions that were once mediated by the mesolimbic dopamine system to patch-based pathways. In addition, the activation of the patch compartment has been shown to be rewarding, as animals will self-stimulate when an electrode is placed in or near the patch compartment, indicating that motivational states may be facilitated by the patch compartment (White and Hiroi, 1998). It is possible that as drug-seeking behaviors become more stimulus-response in nature that patch-based circuits in the dorsolateral striatum participate by conveying and assigning limbic information about internal and motivational states to habit formation. Thus, the patch-based limbic circuits that traverse the striatum, particularly in the dorsolateral region may contribute to behaviors that are linked to motivational states, with the patch compartment potentially serving as a node of contact between the limbic system and basal ganglia whereby internally driven states can affect basal-ganglia mediated adaptive behavioral functions.

It is important to note that the enhanced activation of the patch compartment relative to the matrix compartment observed following METH-mediated CPP is the result of an increase in c-Fos levels in the patch compartment coupled with a down-regulation of c-Fos levels in the matrix compartment. These findings are in line with previous work where a down-regulation of c-Fos activity in the matrix compartment was observed repeated exposure to psychostimulants contributing to a patch-enhanced pattern of activation (Canales and Graybiel, 2000). It is thought that repeated exposure to psychostimulants leads to long-term depression (LTD) in the matrix compartment, although this hypothesis has not been explored (Canales, 2005; Canales and Graybiel, 2000; Graybiel et al., 2000). The sensorimotor cortex, which sends projections primarily to the matrix compartment is strongly activated by psychostimulant treatment (Canales and Graybiel, 2000; O'Dell and Marshall, 2000). It is possible that a progressive depression of glutamatergic corticostriatal inputs could contribute to LTD in the matrix compartment, which may be reflected in the down-regulation of c-Fos activity in this region (Canales and Graybiel, 2000; O'Dell and Marshall, 2000). Recent work indicates that μ opioid receptors in the patch compartment may also modulate neuroplasticity in the matrix compartment. Miura and co-workers found that activation of patch-based μ opioid receptors inhibits presynaptic GABAergic release only in the patch compartment (Miura et al., 2008; Miura et al., 2007). The source of these μ -sensitive GABA inputs are most likely μ opioid receptor-expressing neurons in the patch compartment that send GABAergic collaterals to neighboring medium spiny neurons and to cholinergic interneurons located on the patch-matrix borders (Czubayko and Plenz, 2002; Tunstall et al., 2002; Venance et al., 2004). Activation of patch-based μ opioid receptors suppresses GABA transmission and releases neighboring medium spiny neurons and cholinergic interneurons from inhibition, which through several mechanisms, leads to a pause in the tonic firing of cholinergic interneurons, and ultimately LTD in the neurons of the matrix compartment (Miura et al., 2008; Miura et al., 2007). However, while our data indicates that c-Fos activity

is decreased in the matrix compartment following METH-mediated CPP, blockade of patch-based μ opioid receptors did not reverse the decrease in c-Fos levels in the matrix. Thus, in the context of METH-mediated CPP, the effects of μ opioid receptor blockade appear to be confined to the neurons of the patch, with little effect on the neurons of the matrix compartment. Clearly, additional studies are needed in order to determine whether LTD occurs in the neurons of the matrix compartment following repeated psychostimulant exposure, and whether patch-based μ opioid receptors contribute to this process.

While μ opioid receptor activation is most often associated with neuronal inhibition (Alreja and Aghajanian, 1993), μ opioid receptor activation may also have excitatory effects on cellular activity, as μ opioid receptor activation can induce mitogen-activated kinase-mediated intracellular cascades, cyclase-response element activity, and c-Fos activity (Bilecki et al., 2004; Shoda et al., 2001). Blockade of patch-based μ opioid receptors may decrease neuronal activation of these neurons, and directly inhibit of c-Fos expression during METH-mediated CPP, and diminish output from patch-based limbic circuits, although the specific circumstances that determine whether the effects of μ opioid receptor activation are excitatory or inhibitory are unclear. Alternatively, patch-based μ opioid receptors may modulate dopamine release within the striatum (Gonzalez-Nicolini et al., 2003). The GABAergic neurons of the patch compartment project exclusively to the dopamine neurons of the SNpc, which project back to the striatum (Fujiyama et al., 2011; Gerfen, 1984; Jimenez-Castellanos and Graybiel, 1987; Tokuno et al., 2002). Blockade of μ opioid receptors may disinhibit the neurons of the patch compartment, leading to increased GABA-mediated inhibition of nigrostriatal neurons, and diminished c-Fos expression. However, this scenario is unlikely as the SNpc sends dopaminergic inputs to both the patch and matrix compartment, which would result in μ opioid receptor-mediated changes in c-Fos in both compartments (Fujiyama et al., 2011; Gerfen, 1984; Jimenez-Castellanos and Graybiel, 1987; Tokuno et al., 2002). In addition, amphetamine-induced dopamine release is non-action potential dependent, and therefore, changes in the neuronal excitability of nigrostriatal neurons by patch-based μ opioid receptor blockade most likely would not impact dopamine release in the striatum (Nomikos et al., 1990). Future studies focusing on the specific mechanisms by which patch-based μ opioid receptors alter the activation of the patch compartment, and modulate nigrostriatal dopamine release will shed light on this issue.

As mentioned above, patch-based neurons send inhibitory projections to dopamine neurons in the SNpc (Fujiyama et al., 2011; Gerfen, 1984; Tokuno et al., 2002). These dopamine neurons typically respond to novel and reinforcing stimuli, such as drugs, thus promoting reinforcement learning (Fiorillo et al., 2003). The inhibitory control of these dopamine neurons by the neurons of the patch compartment may prevent dopamine release in response to expected reinforcers, which could lead to the development of well-learned behaviors that become more dependent on internal reinforcing states, and less dependent on external stimuli for its maintenance (Canales, 2005). Furthermore, electrical activation of the patch compartment *in vivo* is rewarding, and can drive instrumental behaviors (White and Hiroi, 1998). Thus, blockade of patch-based μ opioid receptors and the resulting decrease in activity of patch compartment neurons may lead to less dependence of well-learned

behaviors (such as METH-mediated CPP) on internal motivational states and diminished reward.

5. Conclusion

Our study is the first to demonstrate that enhanced relative activation of patch-based circuits is observed following METH-mediated CPP, and that blockade of patch-based μ opioid receptors can reduce this effect. Our data also showed that the patch-enhanced c-Fos activation was due in part to a down-regulation of c-Fos activation in the matrix compartment following METH-mediated CPP, suggesting that reward processes could be the result of preferential activation of striatal pathways through the patch compartment that contain information about emotion and motivation, while the activity in striatal pathways through the matrix compartment that mediate behavioral flexibility, leading to behaviors that are inflexible and dependent on internal states. Together, these data indicate that preferential activation of limbic-associated patch-based circuits is necessary for the rewarding effects of METH, and that patch-based μ opioid receptors modulate the activity of the neurons of the patch compartment, leading to diminished METH-mediated reward.

Acknowledgments

This work was funded by NIH grant (DA025303) and Mercer University Provost's Seed Grant, awarded to KAH.

Literature Cited

- Adams DH, Hanson GR, Keefe KA. Distinct effects of methamphetamine and cocaine on preprodynorphin messenger RNA in rat striatal patch and matrix. *J Neurochem*. 2003; 84:87–93. [PubMed: 12485404]
- Aliane V, Perez S, Nieoullon A, Deniau JM, Kemel ML. Cocaine-induced stereotypy is linked to an imbalance between the medial prefrontal and sensorimotor circuits of the basal ganglia. *Eur J Neurosci*. 2009; 30:1269–1279. [PubMed: 19769590]
- Alreja M, Aghajanian GK. Opiates suppress a resting sodium-dependent inward current and activate an outward potassium current in locus coeruleus neurons. *J Neurosci*. 1993; 13:3525–3532. [PubMed: 8393484]
- Berke JD, Hyman SE. Addiction, dopamine, and the molecular mechanisms of memory. *Neuron*. 2000; 25:515–532. [PubMed: 10774721]
- Bilecki W, Wawrzczak-Bargiela A, Przewlocki R. Activation of AP-1 and CRE-dependent gene expression via mu-opioid receptor. *J Neurochem*. 2004; 90:874–882. [PubMed: 15287893]
- Canales JJ. Stimulant-induced adaptations in neostriatal matrix and striosome systems: transiting from instrumental responding to habitual behavior in drug addiction. *Neurobiol Learn Mem*. 2005; 83:93–103. [PubMed: 15721792]
- Canales JJ, Graybiel AM. A measure of striatal function predicts motor stereotypy. *Nat Neurosci*. 2000; 3:377–383. [PubMed: 10725928]
- Crittenden JR, Graybiel AM. Basal Ganglia disorders associated with imbalances in the striatal striosome and matrix compartments. *Frontiers in neuroanatomy*. 2011; 5:59. [PubMed: 21941467]
- Czubayko U, Plenz D. Fast synaptic transmission between striatal spiny projection neurons. *Proc Natl Acad Sci U S A*. 2002; 99:15764–15769. [PubMed: 12438690]
- Fiorillo CD, Tobler PN, Schultz W. Discrete coding of reward probability and uncertainty by dopamine neurons. *Science*. 2003; 299:1898–1902. [PubMed: 12649484]
- Fujiyama F, Sohn J, Nakano T, Furuta T, Nakamura KC, Matsuda W, Kaneko T. Exclusive and common targets of neostriatofugal projections of rat striosome neurons: a single neuron-tracing study using a viral vector. *Eur J Neurosci*. 2011; 33:668–677. [PubMed: 21314848]

- Gerfen CR. The neostriatal mosaic: compartmentalization of corticostriatal input and striatonigral output systems. *Nature*. 1984; 311:461–464. [PubMed: 6207434]
- Gerfen CR. The neostriatal mosaic: striatal patch-matrix organization is related to cortical lamination. *Science*. 1989; 246:385–388. [PubMed: 2799392]
- Gerfen CR, Baimbridge KG, Miller JJ. The neostriatal mosaic: compartmental distribution of calcium-binding protein and parvalbumin in the basal ganglia of the rat and monkey. *Proc Natl Acad Sci U S A*. 1985; 82:8780–8784. [PubMed: 3909155]
- Gonzalez-Nicolini MV, Berglind W, Cole KS, Keogh CL, McGinty JF. Local mu and delta opioid receptors regulate amphetamine-induced behavior and neuropeptide mRNA in the striatum. *Neuroscience*. 2003; 121:387–398. [PubMed: 14521997]
- Graybiel AM, Canales JJ, Capper-Loup C. Levodopa-induced dyskinesias and dopamine-dependent stereotypies: a new hypothesis. *Trends Neurosci*. 2000; 23:S71–S77. [PubMed: 11052223]
- Graybiel AM, Moratalla R, Robertson HA. Amphetamine and cocaine induce drug-specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum. *Proc Natl Acad Sci U S A*. 1990; 87:6912–6916. [PubMed: 2118661]
- Haber SN, Fudge JL, McFarland NR. Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. *J Neurosci*. 2000; 20:2369–2382. [PubMed: 10704511]
- Herkenham M, Pert CB. Mosaic distribution of opiate receptors, parafascicular projections and acetylcholinesterase in rat striatum. *Nature*. 1981; 291:415–418. [PubMed: 6165892]
- Horner KA, Hebbard JC, Logan AS, Vanchipurakel GA, Gilbert YE. Activation of mu opioid receptors in the striatum differentially augments methamphetamine-induced gene expression and enhances stereotypic behavior. *J Neurochem*. 2012; 120:779–794. [PubMed: 22150526]
- Horner KA, Keefe KA. Regulation of psychostimulant-induced preprodynorphin, *c-fos* and *zif/268* messenger RNA expression in the rat dorsal striatum by mu opioid receptor blockade. *Eur J Pharmacol*. 2006; 532:61–73. [PubMed: 16443216]
- Horner KA, Noble ES, Gilbert YE. Methamphetamine-induced stereotypy correlates negatively with patch-enhanced prodynorphin and arc mRNA expression in the rat caudate putamen: the role of mu opioid receptor activation. *Pharmacol Biochem Behav*. 2010; 95:410–421. [PubMed: 20298714]
- Hyman SE, Malenka RC. Addiction and the brain: the neurobiology of compulsion and its persistence. *Nat Rev Neurosci*. 2001; 2:695–703. [PubMed: 11584307]
- Jedynak JP, Cameron CM, Robinson TE. Repeated methamphetamine administration differentially alters fos expression in caudate-putamen patch and matrix compartments and nucleus accumbens. *PloS one*. 2012; 7:e34227. [PubMed: 22514626]
- Jimenez-Castellanos J, Graybiel AM. Subdivisions of the dopamine-containing A8-A9-A10 complex identified by their differential mesostriatal innervation of striosomes and extrastriosomal matrix. *Neuroscience*. 1987; 23:223–242. [PubMed: 3683862]
- Miura M, Masuda M, Aosaki T. Roles of micro-opioid receptors in GABAergic synaptic transmission in the striosome and matrix compartments of the striatum. *Mol Neurobiol*. 2008; 37:104–115. [PubMed: 18473190]
- Miura M, Saino-Saito S, Masuda M, Kobayashi K, Aosaki T. Compartment-specific modulation of GABAergic synaptic transmission by mu-opioid receptor in the mouse striatum with green fluorescent protein-expressing dopamine islands. *J Neurosci*. 2007; 27:9721–9728. [PubMed: 17804632]
- Murray RC, Gilbert YE, Logan AS, Hebbard JC, Horner KA. Striatal patch compartment lesions alter methamphetamine-induced behavior and immediate early gene expression in the striatum, substantia nigra and frontal cortex. *Brain structure & function*. 2014; 219:1213–1229. [PubMed: 23625147]
- Murray RC, Logan MC, Horner KA. Striatal patch compartment lesions reduce stereotypy following repeated cocaine administration. *Brain Res*. 2015; 1618:286–298. [PubMed: 26100338]
- Natarajan R, Yamamoto BK. The Basal Ganglia as a Substrate for the Multiple Actions of Amphetamines. *Basal Ganglia*. 2011; 1:49–57. [PubMed: 21804952]

- Nomikos GG, Damsma G, Wenkstern D, Fibiger HC. In vivo characterization of locally applied dopamine uptake inhibitors by striatal microdialysis. *Synapse*. 1990; 6:106–112. [PubMed: 1697988]
- Nygaard SK, Klambatsen A, Balouch B, Quinones-Jenab V, Jenab S. Region and context-specific intracellular responses associated with cocaine-induced conditioned place preference expression. *Neuroscience*. 2015; 287:1–8. [PubMed: 25522720]
- O'Dell SJ, Marshall JF. Repeated administration of methamphetamine damages cells in the somatosensory cortex: overlap with cytochrome oxidase-rich barrels. *Synapse*. 2000; 37:32–37. [PubMed: 10842349]
- Paxinos, G., Watson, C. *The Rat Brain in Stereotaxic Coordinates*. San Diego, CA: Elsevier Academic Press; 2005.
- Pert CB, Kuhar M, Snyder SH. Opiate receptor: autoradiographic localization in the rat brain. *Proc Natl Acad Sci USA*. 1976; 73:3729–3733. [PubMed: 185626]
- Robbins TW, Everitt BJ. Limbic-striatal memory systems and drug addiction. *Neurobiol Learn Mem*. 2002; 78:625–636. [PubMed: 12559840]
- Saka E, Goodrich C, Harlan P, Madras BK, Graybiel AM. Repetitive behaviors in monkeys are linked to specific striatal activation patterns. *J Neurosci*. 2004; 24:7557–7565. [PubMed: 15329403]
- Schroeder JA, Hummel M, Simpson AD, Sheikh R, Soderman AR, Unterwald EM. A role for mu opioid receptors in cocaine-induced activity, sensitization, and reward in the rat. *Psychopharmacology (Berl)*. 2007; 195:265–272. [PubMed: 17687547]
- Shoda T, Fukuda K, Uga H, Mima H, Morikawa H. Activation of mu-opioid receptor induces expression of c-fos and junB via mitogen-activated protein kinase cascade. *Anesthesiology*. 2001; 95:983–989. [PubMed: 11605942]
- Suzuki T, Misawa M. Sertindole antagonizes morphine-, cocaine-, and methamphetamine-induced place preference in the rat. *Life sciences*. 1995; 57:1277–1284. [PubMed: 7674819]
- Tokuno H, Chiken S, Kametani K, Moriizumi T. Efferent projections from the striatal patch compartment: anterograde degeneration after selective ablation of neurons expressing mu-opioid receptor in rats. *Neurosci Lett*. 2002; 332:5–8. [PubMed: 12377371]
- Tunstall MJ, Oorschot DE, Kean A, Wickens JR. Inhibitory interactions between spiny projection neurons in the rat striatum. *J Neurophysiol*. 2002; 88:1263–1269. [PubMed: 12205147]
- Vanderschuren LJ, Kalivas PW. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. *Psychopharmacology (Berl)*. 2000; 151:99–120. [PubMed: 10972458]
- Venance L, Glowinski J, Giaume C. Electrical and chemical transmission between striatal GABAergic output neurones in rat brain slices. *J Physiol*. 2004; 559:215–230. [PubMed: 15235091]
- Voorn P, Vanderschuren LJ, Groenewegen HJ, Robbins TW, Pennartz CM. Putting a spin on the dorsal-ventral divide of the striatum. *Trends Neurosci*. 2004; 27:468–474. [PubMed: 15271494]
- Wang JQ, Smith AJ, McGinty JF. A single injection of amphetamine or methamphetamine induces dynamic alterations in *c-fos*, *zif/268* and preprodynorphin messenger RNA expression in the rat forebrain. *Neuroscience*. 1995; 68:83–95. [PubMed: 7477938]
- White NM, Hiroi N. Preferential localization of self-stimulation sites in striosomes/patches in the rat striatum. *Proc Natl Acad Sci U S A*. 1998; 95:6486–6491. [PubMed: 9600993]
- Yin HH, Knowlton BJ, Balleine BW. Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. *Eur J Neurosci*. 2004; 19:181–189. [PubMed: 14750976]

Fig. 1.

Effects of intrastriatal infusion of CTAP (0.8 $\mu\text{g}/\mu\text{l}$) on METH-mediated CPP. Values are expressed as the mean ($\pm$ S.E.M.) number of seconds spent in the drug-paired (initially non-preferred) chamber post-conditioning the minus the number of seconds spent in the initially preferred chamber, prior to conditioning. Blockade of μ opioid receptors on the neurons of the patch compartment with CTAP significantly attenuated the development of METH-induced CPP. Neither CTAP nor VEH alone induced significant place preference or aversion. * P =significantly different from VEH-pretreated, saline-treated group; + p =significantly different from VEH-pretreated, METH-treated group.

Fig. 2. Effects of intrastratial infusion of CTAP on c-Fos immunoreactivity in the patch and matrix compartments following METH-mediated CPP. Representative photomicrographs showing μ opioid receptor and c-Fos immunoreactivity in adjacent sections that are 12 μ m apart. μ opioid receptor immunoreactivity was used to delineate the patch from the matrix compartments, as μ opioid receptors are preferentially expressed in the patch compartment. Scale bar=100 μ m.

Fig. 3. Quantitative analysis of c-Fos immunoreactivity in the patch (A) and matrix (B) compartments of the striatum following intrastratial infusion of CTAP and METH-mediated CPP. The patch or matrix compartment was outlined using adjacent sections labeled for μ opioid receptors, and the region of interest superimposed over the region of interest on c-Fos labeled sections, and the number c-Fos-immunoreactive particles counted within the region of interest. C-Fos immunoreactivity in the patch compartment was increased following METH-induced CPP, an effect that was reversed by pretreatment with CTAP. In the matrix

compartment, there was a significant main effect of treatment following METH treatment, as c-Fos immunoreactivity was decreased in the matrix compartment following METH-mediated CPP, regardless of the pretreatment. Data are presented as the percentage of the number of c-Fos-labeled particles/mm² in VEH-pretreated control animals ($\pm$ S.E.M.). **P*=significantly different from VEH-pretreated, SAL-treated group; +*p*=significantly different from VEH-pretreated, METH-treated group.

Fig. 4. Effects of CTAP pretreatment on the ratio of patch-to-matrix c-Fos immunoreactivity in the striatum following METH-induced CPP. The ratio of patch-to-matrix c-Fos immunoreactivity was significantly increased following METH-induced CPP, and effect that was attenuated by pretreatment with intrastrially infused CTAP. **P*=significantly different from VEH-pretreated, SAL-treated group; +*P*=significantly different from VEH-pretreated, METH-treated group.

Table 1

Locomotor activity during preference testing.

Pretreatment/Treatment	Distance Traveled (m)
VEH/SAL	347 ± 42
VEH/METH	425 ± 32
CTAP/SAL	297 ± 21
CTAP/METH	318 ± 53

Data are shown as the mean ($\pm$ S.E.M.) distance traveled (in meters) during the preference test. There was no significant difference in the distance traveled between any of the groups.