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ORIGINAL ARTICLE

Activation of mu opioid receptors in the striatum differentially augments methamphetamine-induced gene expression and enhances stereotypic behavior

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Abstract

Mu opioid receptors are densely expressed in the patch compartment of striatum and contribute to methamphetamine-induced patch-enhanced gene expression and stereotypy. To further elucidate the role of mu opioid receptor activation in these phenomena, we examined whether activation of mu opioid receptors would enhance methamphetamine-induced stereotypy and prodynorphin, *c-fos*, *arc* and *zif/268* expression in the patch and/or matrix compartments of striatum, as well as the impact of mu opioid receptor activation on the relationship between patch-enhanced gene expression and stereotypy. Male Sprague–Dawley rats were intrastriatally infused with D-Ala(2)-N-Me-Phe(4),Gly(5)-ol]enkephalin (DAMGO; 1 μg/μL), treated with methamphetamine (0.5 mg/kg) and killed at 45 min or 2 h later. DAMGO augmented methamphetamine-induced *zif/268* mRNA expression in the patch and matrix compartments, while prodynorphin expression was increased in the dorsolateral patch compartment. DAMGO pre-treatment did not affect methamphetamine-induced *arc* and *c-fos* expression. DAMGO enhanced methamphetamine-induced stereotypy and resulted in greater patch versus matrix expression of prodynorphin in the dorsolateral striatum, leading to a negative correlation between the two. These findings indicate that mu opioid receptors contribute to methamphetamine-induced stereotypy, but can differentially influence the genomic responses to methamphetamine. These data also suggest that prodynorphin may offset the overstimulation of striatal neurons by methamphetamine.

Keywords: behavior, caudate putamen, immediate early gene, opioid, psychostimulant.

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Psychostimulants modify basal ganglia function and induce significant changes in behavior as a result of alterations in neuropeptide and immediate early gene expression in the striatum (Hanson *et al.* 1987; Moratalla *et al.* 1992; Wang and McGinty 1995; Wang *et al.* 1995; Harlan and Garcia 1998; Canales and Graybiel 2000; Tan *et al.* 2000; Adams *et al.* 2003; Gonzalez-Nicolini *et al.* 2003; Horner and Keefe 2006; Horner *et al.* 2010). For example, treatment with methamphetamine induces a higher degree of dynorphin, *c-fos*, *arc* and *zif/268* mRNA expression in the patch (striosome) compartment relative to the surrounding matrix compartment of rostral striatum, resulting in a patch-enhanced pattern of gene expression (Wang *et al.* 1995; Adams *et al.* 2003; Horner and Keefe 2006; Horner *et al.* 2010). The immediate early genes *zif/268* and *c-fos* code for transcription factors that act on downstream target genes, including those encoding neuropeptides in the striatum, whereas *arc* mRNA is trafficked to activated synapses (Milbrandt 1987; Cole *et al.* 1995; Lyford *et al.* 1995; Steward *et al.* 1998; Steward and Worley 2001). However, dynorphin could serve as a negative feedback mechanism to regulate striatal neuron function, possibly in response to overstimulation of striatal neurons by psychostimulants

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Abbreviations used: BSA, bovine serum albumin; CRE, cyclic AMP response element; DAMGO, D-Ala(2)-*N*-Me-Phe(4),Gly(5)-ol]enkephalin; ERK, extracellular signal-regulated kinase; PBS, phosphate-buffered saline; SAL, saline; SRE, serum response element; SSC, saline-sodium citrate; VEH, vehicle.

(Steiner and Gerfen 1998; Horner *et al.* 2010). Thus, activation of *arc, c-fos* and/or *zif/268* may be an initial step in a chain of transcriptional events that impact long-term plasticity in neurons and along with dynorphin, could ultimately influence the behavioral responses to treatment with methamphetamine.

It is thought that psychostimulant-induced stereotypy may be related to the induction of patch-enhanced gene expression in the rostral striatum (Canales and Graybiel 2000; Graybiel and Canales 2000; Graybiel *et al.* 2000; Canales 2005). The neurons of the patch compartment receive inputs from limbic-related areas, such as prelimbic cortex and based upon its connections with periallocortical regions, possess circuitry that limbic in nature, whereas neurons in the matrix compartment receive inputs from sensorimotor and association cortices, and because of its connections with neocortex, possesses a circuitry that is less limbic in nature (Gerfen 1984, 1989, 1992b; Bolam *et al.* 1988; Ragsdale and Graybiel 1988; Wang and Pickel 1998). It has been suggested that enhanced activity of patch-based, limbic-associated circuits, relative to the matrix-based, motor-associated circuits may be related to inflexible, internally driven behaviors, such as stereotypy (Canales and Graybiel 2000; Graybiel and Canales 2000; Canales 2005). Yet, the exact nature of the relationship between enhanced activation of the patch compartment relative to the matrix compartment and stereotypic behavior following psychostimulant treatment is not completely understood, as previous studies have shown positive, negative, or no correlation between patch-enhanced activity and psychostimulant-induced stereotypy (Canales and Graybiel 2000; Saka *et al.* 2002; Glickstein and Schmauss 2004; Horner *et al.* 2010).

However, despite the disparate findings regarding the precise relationship between patch-enhanced activity and psychostimulant-induced stereotypy, several lines of evidence point to a role for the activation of mu opioid receptors in psychostimulant-induced patch-enhanced gene expression, as well as stereotypic behavior. First, mu opioid receptors are expressed in high density by the neurons of the patch compartment, and may be located extrasynaptically on dendrites where they are co-localized with tyrosine hydroxylase-containing afferents, or on dendritic spines, where they receive asymmetric inputs from prefrontal corticostriatal afferents (Pert *et al.* 1976; Herkenham and Pert 1981; Tempel and Zukin 1987; Wang *et al.* 1996; Wang and Pickel 1998). Thus, mu opioid receptors are anatomically positioned to influence gene expression within the neurons of the patch compartment both directly and indirectly through modulation of post-synaptic responses to corticostriatal and nigrostriatal activation (Wang *et al.* 1997; Wang and Pickel 1998). Second, blockade of mu opioid receptors attenuates psychostimulant-induced dynorphin expression in the patch compartment of rostral striatum, and prevents patch-enhanced expression of dynorphin in the dorsolateral striatum by methamphetamine as a result of a decrease in the ratio of patch-to-matrix mRNA expression in this region (Horner and Keefe 2006; Horner *et al.* 2010). Finally, blockade of striatal mu opioid receptors can reduce methamphetamine-induced stereotypic behavior, while pre-treatment with the mu opioid receptor agonist morphine has been shown to enhance amphetamine-induced stereotypy (Woo *et al.* 1985; Horner *et al.* 2010).

Together, these data indicate that striatal mu opioid receptor activation contributes to methamphetamine-induced gene expression and behavior, but also suggest that methamphetamine-induced stereotypic behavior and/or gene expression could be intensified if striatal mu opioid receptors are activated during treatment with a low dose of methamphetamine that induces relatively low levels stereotypic behavior and gene expression. However, whether activation of striatal mu opioid receptors in combination with a low dose of methamphetamine results in an increase in dynorphin or immediate early gene mRNA expression in the patch relative to the matrix compartment or increased stereotypy is presently unknown. Thus, the purpose of the present study was to determine whether activation of striatal mu opioid receptors followed by treatment with a low dose of methamphetamine results in relatively higher levels of dynorphin and immediate early gene mRNA expression in the patch versus matrix compartments striatum, and whether mu opioid receptor activation increases the severity of stereotypic behavior. In addition, we examined whether activation of mu opioid receptors altered the correlation between the ratio of mRNA expression in the patch versus matrix compartments and stereotypy.

Methods

Animals and surgery

Male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN, USA), weighing 250–350 g were used in all experiments. Rats were housed in groups of four in plastic cages in a temperature-controlled room. Rats were on a 14 : 10 h light/dark cycle and had 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 steps were taken to minimize any suffering that might occur during our procedures.

Five to seven days prior to the experiment, rats were implanted bilaterally in the rostral striatum (coordinates relative to bregma: AP +1.68 to +2.16 mm, ML ±2.6 mm, DV −3.5 mm) with 26-gauge guide cannulae (Plastics One, Roanoke, VA, USA) that were 3.5 mm in length that were kept patent with 31-gauge obturators that were also 3.5 mm in length, as previously described (Horner *et al.* 2010). The day before the experiment the obturators were removed and replaced with dummy cannulae that extended 2.0 mm beyond the guide cannulae, to minimize acute tissue damage and spurious immediate early gene expression during the experiment

(Keefe and Gerfen 1995; Adams *et al.* 2000; Horner *et al.* 2010). The day of the experiment, 31-gauge injection cannulae that extended 1.5 mm beyond the guide were inserted into the guide cannulae and a 1-μL volume of buffered artificial CSF (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) or the mu opioid receptor agonist, D-Ala(2)-*N*-Me-Phe(4),Gly(5)-ol]enkephalin (DAMGO; 1 μg/μL, Woo *et al.* 1985) was administered bilaterally at a rate of 0.1 μL/min to the freely moving animal. After each infusion, the injection cannulae were left in place for 5 min to minimize fluid back flow through the cannulae. Only animals whose cannulae were in the rostral striatum were included in subsequent analyses.

Experimental design and behavior

Twenty-four hours prior to the experiment, animals were habituated to plexiglass activity chambers (Frankel *et al.* 2007; Horner *et al.* 2010) by placing them in the chambers for 60 min, giving them sham injections and returning them to the chambers for either 45 min or 2 h. The next day, animals were placed in the chambers for 60 min, after which time they were bilaterally infused with either artificial CSF or 1 μg/μL DAMGO, as described above. The animals were then injected with methamphetamine (0.5 mg/kg, s.c., a dose determined by dose–response pilot studies performed in our laboratory) or saline and returned to the activity chambers for 45 min or 2 h, during which time the behavior was digitally recorded for *post hoc* analyses. During the *post hoc* analyses, each animal was observed for 1 min every 5 min for the entire 45 min or 2 h observation period after the injection of methamphetamine or saline by an observer blind to the experimental conditions (Horner *et al.* 2010). Stereotypy was rated on a scale of 1–10, with 10 representing the highest degree of the response, and scores were generated as previously described (Canales and Graybiel 2000; Horner *et al.* 2010), by averaging the scores from four behavioral dimensions: repetitiveness/flexibility, frequency, duration and spatial distribution of the motor response.

In situ hybridization histochemistry

In situ hybridization histochemistry for detection of changes in mRNA expression was performed as previously described (Horner *et al.* 2010). Forty-five minutes (for immediate early gene expression; Horner and Keefe 2006) or 2 h (for prodynorphin expression; Wang *et al.* 1995) after treatment with methamphetamine or saline, rats were killed by exposure to CO_2 for 1 min followed by decapitation. The brains were rapidly harvested, quick-frozen in isopentane on dry ice and stored at −80°C until they were cut into 12-μm sections on a cryostat (Minotome Plus, Triangle Biomedical Sciences, Durham, NC, USA). Sections were thaw-mounted onto Superfrost slides (VWR, Westchester, PA, USA) and stored at −20°C. Slides from all animals were then post-fixed in 4% paraformaldehyde/0.9% NaCl, acetylated in fresh 0.25% acetic anhydride in 0.1 M triethanolamine/0.9% NaCl (pH 8.0), dehydrated in alcohol, delipidated in chloroform and gradually rehydrated in a descending series of alcohol concentrations. Slides were air-dried and stored at −20°C.

Oligonucleotide probes (GeneDetect, Bradenton, FL, USA) complementary to bases 762–809 of prodynorphin (Civelli *et al.* 1985), 1227–1274 of *c-fos* (Curran *et al.* 1987), 377–424 of *arc* (Lyford *et al.* 1995) or 355–399 of *zif/268* (Milbrandt 1987) mRNA were end-labeled with [^{33}P]-dATP (Perkin Elmer NEN, Wellesley, MA, USA). Each probe was diluted in hybridization buffer (0.6 M sodium chloride, 80 mM Tris, 4 mM EDTA, 0.1% w/v sodium pyrophosphate, 10% w/v dextran, 0.2% w/v lauryl sulfate, 0.5 mg/mL heparin, 50% formamide) and 90 μL of the probe in hybridization buffer was applied to each slide and covered with glass coverslips. Slides were hybridized overnight in humid chambers at 37°C, followed by four washes in 1× saline-sodium citrate (SSC; 0.15 M NaCl, 0.015 M sodium citrate, pH 7.2) at 25°C and then three washes in 2× SSC with 50% (v/v) formamide at 42°C. Slides were washed twice in 1× SSC at 25°C, dipped in deionized H_2O and air-dried. All labeled slides were apposed to X-ray film (Kodak Biomax MR film; Kodak Company, Rochester, NY, USA) for approximately 30 days.

Mu opioid receptor immunohistochemistry

To anatomically distinguish the patch and matrix compartments of striatum, immunohistochemistry for mu opioid receptors was performed on serial, 12-μm sections through the striatum that were adjacent to those used for *in situ* hybridization, as previously described (Horner *et al.* 2005, 2010; Horner and Keefe 2006). Briefly, sections were post-fixed in 4% paraformaldehyde/0.9% NaCl, rinsed three times in 0.1 M phosphate-buffered saline (PBS) and blocked with 10% bovine serum albumin (BSA)/0.3% Triton X-100 (TX)/0.1 M PBS for 2 h followed by overnight incubation at 4°C with a polyclonal antibody for the mu opioid receptor (Immunostar, Hudson, WI, USA), diluted in 1 : 1000 in 0.3% TX/0.1M PBS/5% BSA. The slides were then washed several times in PBS and incubated for 2 h at 25°C in biotinylated goat anti-rabbit IgG antiserum (Vector Laboratories, Burlingame, CA, USA) diluted 1 : 200 in 0.1M 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 H_2O, dehydrated in a series of alcohols and coverslipped out of xylene.

Film analysis

Film autoradiograms were analyzed using the image analysis program ImageJ (National Institutes of Health; http://rsb.info.nih.gov/ij), as previously described (Adams *et al.* 2003; Horner *et al.* 2005, 2010; Horner and Keefe 2006). Briefly, images from five to eight animals in each treatment group were analyzed for each mRNA and one section per animal was analyzed for each region of interest examined. Measurements were made according to the coordinates of Paxinos and Watson (2005) in the left hemisphere of the rostral striatum (approximately +1.7 mm anterior to bregma). The average gray value of the white matter overlying the structure being measured was subtracted from the average gray value of the region of interest to correct for background labeling.

To distinguish the patch and matrix compartments of the striatum, sections adjacent to those used for *in situ* hybridization for prodynorphin, *c-fos*, *arc* or *zif/268* mRNA were processed for mu opioid receptor immunohistochemistry, as described above. Measurements were made in the patch and matrix of the rostral striatum, as previously described (Adams *et al.* 2003; Horner *et al.* 2005, 2010; Horner and Keefe 2006), and encompassed four sub-regions: dorsolateral, dorsomedial, ventrolateral and ventromedial striatum

(Adams *et al.* 2001; Horner *et al.* 2010). Immunohistochemically labeled sections were captured at the same magnification as the *in situ* hybridization-labeled sections. Patches of mu opioid receptor immunoreactivity were outlined using the ImageJ software and superimposed over corresponding areas on the *in situ* hybridization-labeled striatal sections and analyzed as described above. Areas where mu opioid receptor immunoreactivity was absent were analyzed as a measure of mRNA expression in the matrix compartment of striatum (Horner *et al.* 2005, 2010; Horner and Keefe 2006). A ratio of patch-to-matrix mRNA expression (Canales and Graybiel 2000; Horner *et al.* 2009, 2010) was calculated for each mRNA, at each survival time, in each of the four sub-regions of the rostral striatum and was accomplished by dividing the average gray value of the patch by the average gray value of the matrix, for each animal in the study.

Statistical analysis

The effects of mu opioid receptor activation on methamphetamine-induced prodynorphin, *c-fos*, *arc* and *zif/268* mRNA expression in the rostral striatum was analyzed using a two-way (pre-treatment × acute treatment) analysis of variance. A separate analysis was performed for the patch and matrix compartments in each of the four sub-regions of the rostral striatum. Behavioral rating data was represented as the area under the curve and was also analyzed using a two-way (pre-treatment × acute treatment) 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 (saline vs. methamphetamine). For the *post hoc* analysis of significant interaction terms, four comparisons were made (vehicle/saline vs. vehicle/methamphetamine; vehicle/saline vs. DAMGO/saline; vehicle/methamphetamine vs. DAMGO/methamphetamine; DAMGO/saline vs. DAMGO/methamphetamine), thus requiring a p-value = 0.0125 (0.05/4) for statistical significance. Correlations between the ratio of patch-to-matrix gene expression in each of the four sub-regions of the rostral striatum for each mRNA and the cumulative stereotypy score for the either the entire 45-min or 2-h observation period, were calculated according to the Spearman method. Statistical significance was set at $p < 0.05$.

Drugs

(±)Methamphetamine hydrochloride was a generous gift from the National Institute on Drug Abuse (Bethesda, MD, USA). Ketamine hydrochloride and xylazine hydrochloride were obtained from Sigma Aldrich (St Louis, MO, USA). Methamphetamine, ketamine and xylazine doses were calculated as the free base and dissolved in normal saline. All drugs were given in a volume of 1 mL/kg. DAMGO was obtained from Sigma Aldrich and dissolved in artificial CSF.

Results

Effects of DAMGO pre-treatment on *arc* mRNA expression in the patch and matrix compartments of striatum, 45 min after treatment with methamphetamine

Expression of *arc* mRNA was homogeneous in appearance in the striatum of both vehicle and DAMGO-pre-treated animals, 45 min after treatment with methamphetamine (Fig. 1a). Two-way analysis of variance of the effects of mu opioid receptor activation on methamphetamine-induced *arc* mRNA expression revealed a significant overall effect of methamphetamine treatment, without significant effects of DAMGO pre-treatment or a pre-treatment × treatment interaction in both the patch and matrix compartments of all four sub-regions of striatum examined. *Post hoc* analyses found that compared with saline-treated controls, methamphetamine significantly increased the *arc* mRNA signal in the patch compartment of dorsolateral ($t = 5.84$; $p < 0.0001$), dorsomedial ($t = 5.20$; $p < 0.0001$), ventrolateral ($t = 8.52$; $p < 0.0001$) and ventromedial ($t = 8.04$; $p < 0.0001$) striatal sub-regions, as well as the matrix compartment of dorsolateral ($t = 5.04$; $p < 0.0001$), dorsolateral ($t = 3.95$; $p = 0.0008$), ventrolateral ($t = 8.92$; $p < 0.0001$) and ventromedial ($t = 8.68$; $p < 0.0001$) striatum (Fig. 1b–e). DAMGO pre-treatment did not significantly alter basal *arc* mRNA expression in the patch or matrix of any sub-region of striatum examined.

Effects of DAMGO pre-treatment on *zif/268* mRNA expression in the patch and matrix compartments of striatum, 45 min after treatment with methamphetamine

In the striatum of vehicle-pre-treated animals, 45 min after treatment with methamphetamine, *zif/268* mRNA expression was diffuse in appearance, while in DAMGO pre-treated animals this effect appeared to be accentuated (Fig. 2a). Two-way analysis of variance of the effects of striatal mu opioid receptor activation on methamphetamine-induced *zif/268* mRNA expression revealed a significant effect of DAMGO pre-treatment, a significant effect of methamphetamine treatment and a significant pre-treatment × treatment interaction in both the patch and matrix compartments of dorsolateral, dorsomedial, and ventrolateral striatum (Fig. 2b–e). *Post hoc* analyses revealed that methamphetamine treatment alone did not significantly increase *zif/268* mRNA expression in the patch compartment of dorsolateral ($t = 2.7$; $p = 0.02$), dorsomedial ($t = 1.8$; $p = 0.10$), ventrolateral ($t = 2.8$; $p = 0.02$) striatum of vehicle-pre-treated animals. However, in DAMGO-pre-treated animals, methamphetamine treatment significantly increased *zif/268* mRNA in the patch compartment of dorsolateral ($t = 5.3$; $p = 0.0005$), dorsomedial ($t = 8.6$; $p < 0.0001$) and ventrolateral ($t = 7.2$; $p < 0.0001$) striatum. In addition, the effect of methamphetamine treatment on *zif/268* mRNA expression in the patch compartment was significantly greater in DAMGO-pre-treated animals than vehicle-pre-treated animals in the dorsolateral ($t = 3.9$; $p = 0.005$) and dorsomedial ($t = 4.9$; $p = 0.001$), but not ventrolateral ($t = 3.0$; $p = 0.02$) striatum. P*ost hoc* analyses also revealed that methamphetamine treatment alone did not significantly increase *zif/268* mRNA expression in the matrix compartment of dorsolateral ($t = 2.1$; $p = 0.07$), dorsomedial ($t = 1.7$; $p = 0.13$) or ventrolateral ($t = 2.7$; $p = 0.02$)

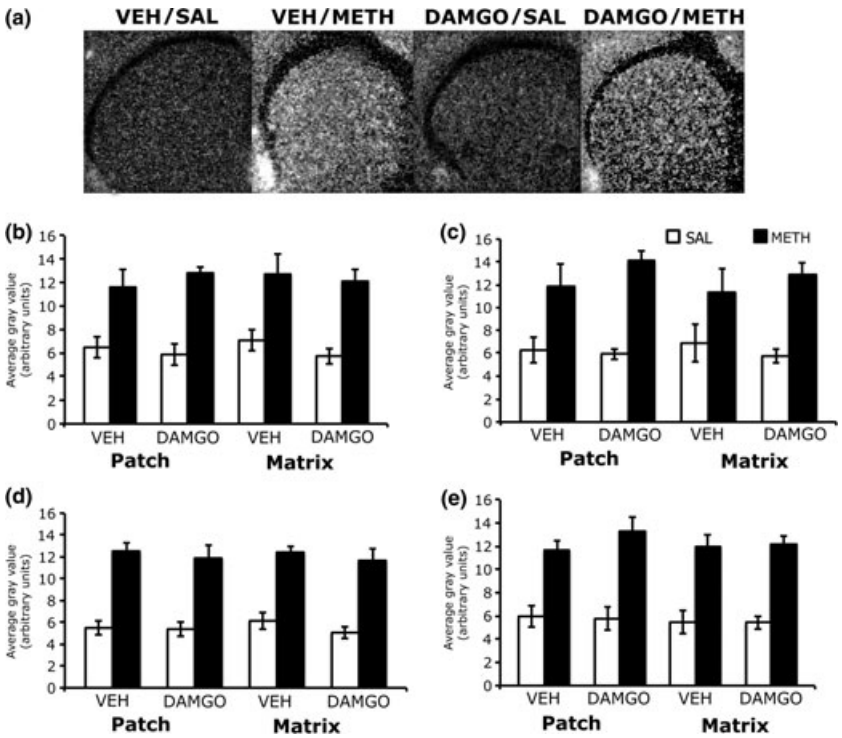

Fig. 1 Effects of DAMGO pre-treatment on methamphetamine-induced *arc* mRNA expression in the rostral striatum, 45 min post-treatment. *In situ* hybridization films (a) showing *arc* mRNA expression in the rostral striatum. Note the homogenous pattern of *arc* mRNA expression in methamphetamine-treated animals, and the similar levels of *arc* mRNA expression between vehicle- and DAMGO-pre-treated methamphetamine-treated animals. Quantitative analysis of *arc* mRNA expression in the patch and matrix compartments of dorsolateral (b), dorsomedial (c), ventrolateral (d) and ventromedial (e) striatum, from rats intrastriatally infused with vehicle or DAMGO (1 μg/μL) 15 min prior to treatment with methamphetamine (0.5 mg/kg). Quantitative values are average gray values (arbitrary units, ± SEM, $n = 5–8$ animals). There was a significant overall main effect of methamphetamine treatment in the patch and matrix compartments of all four sub-regions of striatum.

striatum in vehicle-pre-treated animals, but did significantly increase *zif/268* mRNA expression in the matrix compartment of dorsolateral ($t = 4.9$; $p = 0.001$), dorsomedial ($t = 6.7$; $p < 0.0001$) and ventrolateral ($t = 8.4$; $p < 0.0001$) striatum in DAMGO-pre-treated animals. In addition, the effect of methamphetamine treatment on *zif/268* mRNA expression in the matrix compartment was significantly greater in DAMGO-pre-treated animals than vehicle-pre-treated animals in the dorsolateral ($t = 3.4$; $p = 0.01$), dorsomedial ($t = 3.3$; $p = 0.01$) and ventrolateral ($t = 3.6$; $p = 0.007$) striatum. In the both the patch and matrix compartments of ventromedial striatum, two-way analysis of variance revealed an overall significant main effect of treatment, but not a significant effect of pre-treatment or a pre-treatment-treatment interaction (Fig. 2e). *Post hoc* analysis showed that methamphetamine significantly increased *zif/268* mRNA expression in both the patch ($t = 7.9$; $p < 0.0001$) and matrix ($t = 6.0$; $p < 0.0001$) compartments of this striatal sub-region. DAMGO pre-treatment did not significantly alter basal *zif/268* mRNA expression in the patch or matrix of any sub-region of striatum examined.

Effects of DAMGO pre-treatment on *c-fos* mRNA expression in the patch and matrix compartments of striatum, 45 min after treatment with methamphetamine

Expression of *c-fos* mRNA appeared to be increased medially in the striatum of both vehicle and DAMGO-pre-treated animals, 45 min after treatment with methamphetamine (Fig. 3a). Two-way analysis of variance of the effects of mu opioid receptor activation on methamphetamine-induced *c-fos* mRNA expression revealed a significant overall effect of methamphetamine treatment, without a significant effect of DAMGO pre-treatment or a pre-treatment-treatment interaction for both the patch and matrix compartments of dorsomedial striatum (Fig. 3c). *Post hoc* analyses found that *c-fos* mRNA expression was significantly increased in the patch ($t = 2.7$; $p = 0.01$) and matrix ($t = 3.2$; $p = 0.005$) compartments within this sub-region following methamphetamine treatment. There was not a significant effect of pre-treatment, treatment or pre-treatment × treatment interaction in the patch or matrix compartment of any other sub-region examined, nor did DAMGO pre-treatment significantly alter basal *c-fos* mRNA expression (Fig. 3b, d and e).

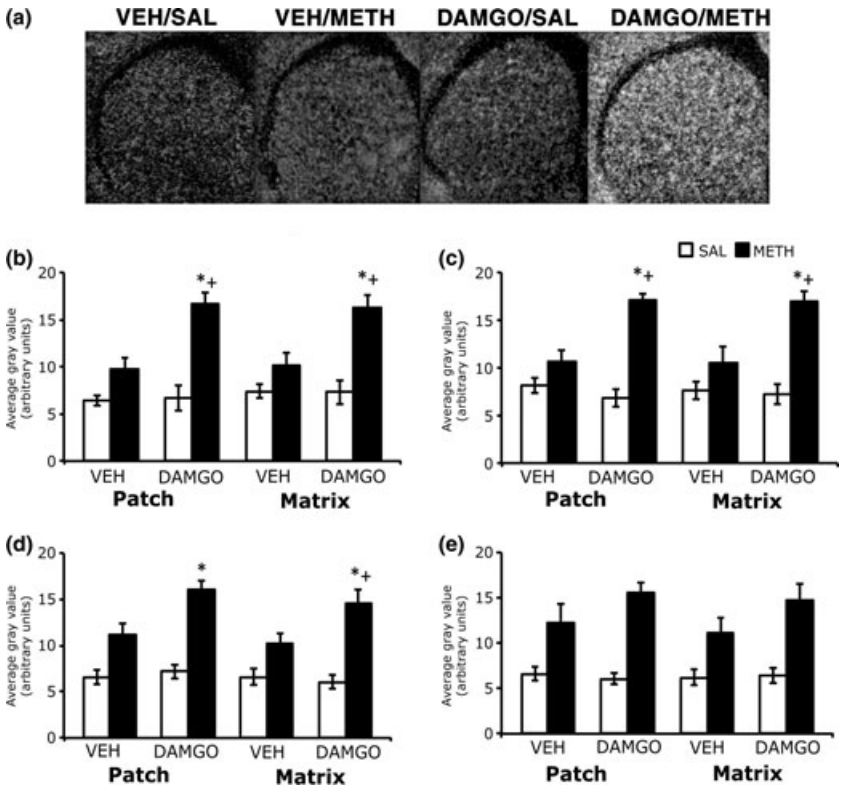

Fig. 2 Effects of DAMGO pre-treatment on methamphetamine-induced *zif/268* mRNA expression in the rostral striatum, 45 min post-treatment. *In situ* hybridization films (a) showing *zif/268* mRNA expression in the rostral striatum. Note the enhanced *zif/268* mRNA expression, as well as the homogenous pattern of expression in DAMGO-pre-treated, methamphetamine-treated animals. Quantitative analysis of *zif/268* mRNA expression in the patch and matrix compartments of dorsolateral (b), dorsomedial (c), ventrolateral (d) and ventromedial (e) striatum, from rats intrastriatally infused with vehicle or DAMGO (1 μg/μL) 15 min prior to treatment with methamphetamine (0.5 mg/kg). Quantitative values are average gray values (arbitrary units, ± SEM, $n = 5–8$ animals). *Significantly different from vehicle-pre-treated control group, $p < 0.05$; $^{+}$Significantly different from vehicle-pre-treated methamphetamine-treated group, $p < 0.05$. There was a significant overall main effect of methamphetamine treatment in the patch and matrix compartments of ventromedial striatum.

Effects of DAMGO pre-treatment on *arc* mRNA expression in the patch and matrix compartments of striatum, 2 h after treatment with methamphetamine

Two-way analysis of the effects of striatal mu opioid receptor activation on methamphetamine-induced *arc* mRNA expression revealed that 2 h following treatment, there was not a significant effect of DAMGO pre-treatment, methamphetamine treatment or significant pre-treatment × treatment interaction in either patch or matrix compartment of any sub-region of striatum examined, nor did DAMGO pre-treatment significantly alter basal *arc* mRNA expression at this time point (data not shown).

Effects of DAMGO pre-treatment on *zif/268* mRNA expression in the patch and matrix compartments of striatum, 2 h after treatment with methamphetamine

In the striatum of vehicle pre-treated animals, 2 h after treatment with methamphetamine, *zif/268* mRNA expression was diffuse in appearance, while in DAMGO pre-treated animals, this effect appeared to be enhanced (Fig. 4a), similar to what was observed 45 min post-methamphetamine treatment. Two-way analysis of variance of the effects of striatal mu opioid receptor activation on methamphetamine-induced *zif/268* mRNA expression revealed a significant effect of DAMGO pre-treatment, a significant effect of methamphetamine treatment, and a significant pre-treatment × treatment interaction in both the patch and matrix compartments of all four sub-regions of striatum (Fig. 4b–e). *Post hoc* analyses revealed that methamphetamine treatment alone did not significantly increase *zif/268* mRNA expression in the dorsolateral ($t = 1.7$; $p = 0.12$), dorsomedial ($t = 0.53$; $p = 0.60$), ventrolateral ($t = 1.5$; $p = 0.16$) or ventromedial ($t = 0.53$; $p = 0.63$) patch compartment of vehicle-pre-treated animals. However, in the patch compartment of DAMGO-pre-treated, methamphetamine-treated animals, *zif/268* mRNA expression was significantly increased in all four striatal sub-regions (dorsolateral, $t = 3.4$; $p = 0.004$; dorsomedial, $t = 3.7$; $p = 0.005$; ventrolateral, $t = 3.9$; $p = 0.003$; ventromedial, $t = 4.8$; $p = 0.0009$). In addition, the effect of methamphetamine treatment on *zif/268* mRNA

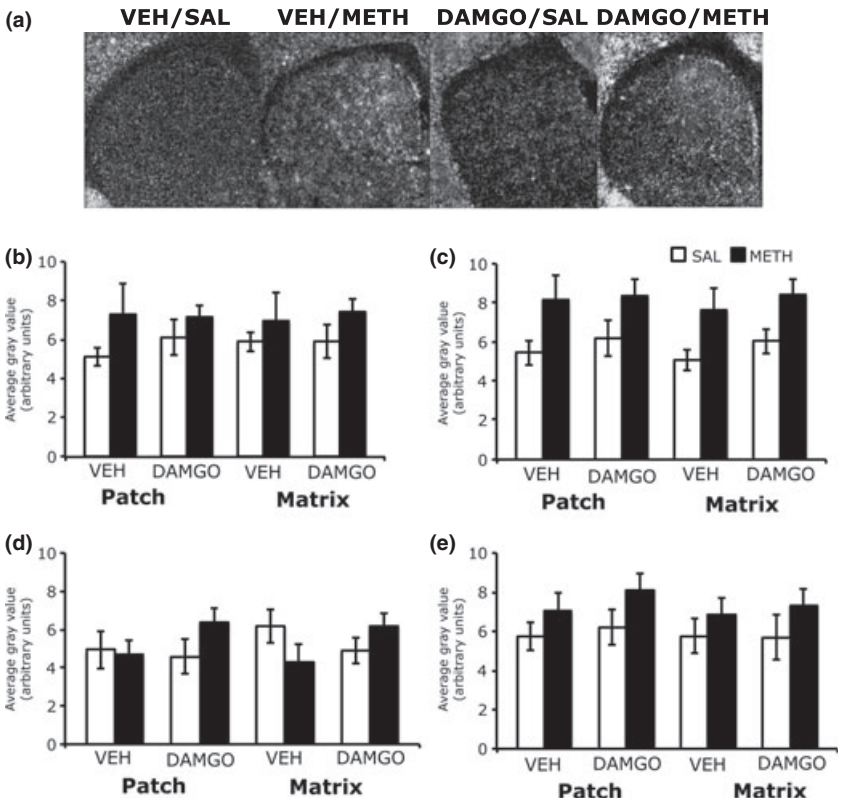

Fig. 3 Effects of DAMGO pre-treatment on methamphetamine-induced *c-fos* mRNA expression in the rostral striatum, 45 min post-treatment. *In situ* hybridization films (a) showing *c-fos* mRNA expression in the rostral striatum. Notice the slight increase in *c-fos* mRNA expression in the dorsomedial aspects of striatum in methamphetamine-treated animals, and the similar levels of *c-fos* mRNA expression between vehicle- and DAMGO-pre-treated methamphetamine-treated animals. Quantitative analysis of *c-fos* mRNA expression in the patch and matrix compartments of dorsolateral (b), dorsomedial (c), ventrolateral (d) and ventromedial (e) striatum, from rats intrastriatally infused with vehicle or DAMGO (1 μg/μL) 15 min prior to treatment with methamphetamine (0.5 mg/kg). Quantitative values are average gray values (arbitrary units, ± SEM, $n = 5$–8 animals). There was a significant overall main effect of methamphetamine treatment in the patch and matrix compartments all four sub-regions of striatum.

expression in the patch compartment was significantly greater in DAMGO-pre-treated animals than vehicle-pre-treated animals in the dorsolateral ($t = 3.6$; $p = 0.004$), dorsomedial ($t = 4.3$; $p = 0.001$), ventrolateral ($t = 4.1$; $p = 0.002$) and ventromedial ($t = 4.6$; $p = 0.0006$) striatum. *Post hoc* analyses revealed that in the matrix compartment, methamphetamine treatment alone did not significantly increase *zif/268* mRNA expression in the dorsolateral ($t = 1.6$; $p = 0.16$), dorsomedial ($t = 1.6$; $p = 0.13$), ventrolateral ($t = 1.0$; $p = 0.36$) or ventromedial ($t = 0.85$; $p = 0.42$) sub-regions of vehicle-pre-treated animals, but in DAMGO pre-treated, methamphetamine-treated animals, *zif/268* mRNA expression was significantly increased in all four sub-regions of striatum (dorsolateral, $t = 7.5$; $p = 0.004$; dorsomedial, $t = 4.9$; $p = 0.0009$; ventromedial, $t = 4.8$; $p = 0.001$; ventrolateral, $t = 3.6$; $p = 0.007$). In addition, the effect of methamphetamine treatment on *zif/268* mRNA expression in the matrix compartment was significantly greater in DAMGO-pre-treated animals than vehicle-pre-treated animals in the dorsolateral ($t = 3.7$; $p = 0.004$), dorsomedial ($t = 4.3$; $p = 0.001$), ventrolateral ($t = 3.6$; $p = 0.007$) and ventromedial ($t = 4.7$; $p = 0.0005$) striatum. DAMGO pre-treatment did not significantly alter basal *zif/268* mRNA expression in the patch or matrix of any sub-region of striatum examined at this time point.

Effects of DAMGO pre-treatment on prodynorphin mRNA expression in the patch and matrix compartments of striatum, 2 h after treatment with methamphetamine

In the striatum of vehicle pre-treated animals, 2 h after treatment with a low dose of methamphetamine, prodynorphin mRNA expression was slightly increased in the patch and matrix compartments of striatum, while in DAMGO pre-treated animals, prodynorphin expression in the patch compartment appeared to be enhanced (Fig. 5a). Two-way analysis of variance of the effects of striatal mu opioid receptor activation on methamphetamine-induced prodynorphin mRNA expression in the patch compartment revealed a significant effect of DAMGO pre-treatment, a significant effect of methamphetamine treatment, and a significant

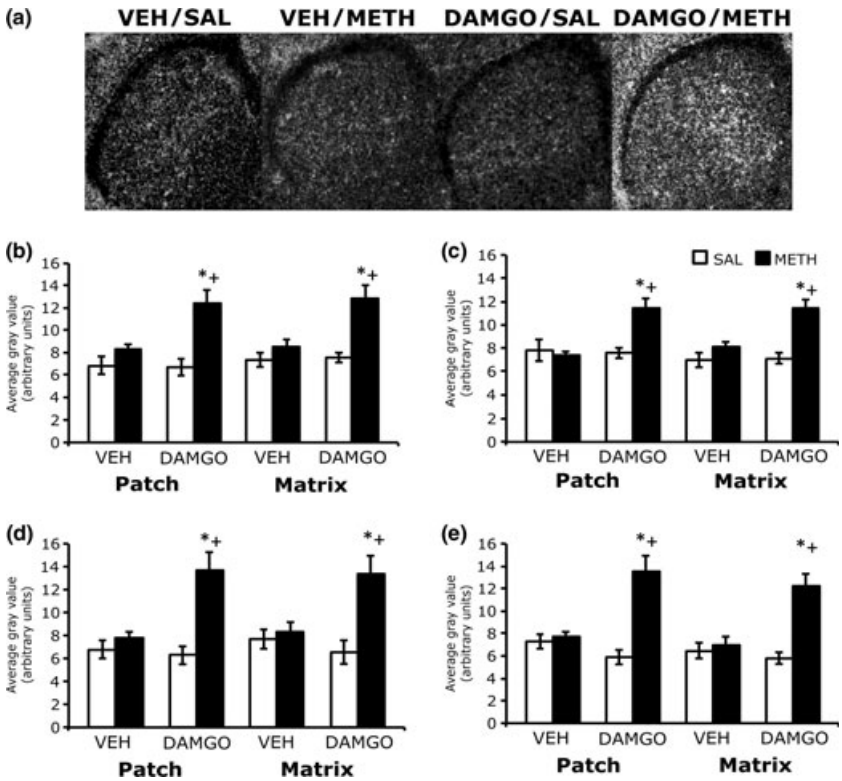

Fig. 4 Effects of DAMGO pre-treatment on methamphetamine-induced *zif/268* mRNA expression in the rostral striatum, 2 h post-treatment. *In situ* hybridization films (a) showing *zif/268* mRNA expression in the rostral striatum. Note the enhanced *zif/268* mRNA expression in DAMGO-pre-treated, methamphetamine-treated animals. Quantitative analysis of *zif/268* mRNA expression in the patch and matrix compartments of dorsolateral (b), dorsomedial (c), ventrolateral (d) and ventromedial (e) striatum, from rats intrastriatally infused with vehicle or DAMGO (1 μg/μL) 15 min prior to treatment with methamphetamine (0.5 mg/kg). Quantitative values are average gray values (arbitrary units, ± SEM, n = 5–8 animals). *Significantly different from vehicle-pre-treated control group, $p < 0.05$; $^{+}$Significantly different from vehicle-pre-treated methamphetamine-treated group, $p < 0.05$.

pre-treatment × treatment interaction in the dorsolateral and dorsomedial sub-regions of striatum (Fig. 5b and c). *Post hoc* analyses revealed that methamphetamine treatment significantly increased prodynorphin mRNA expression in dorsolateral (t = 8.1; $p < 0.0001$), but not dorsomedial (t = 1.2; p = 0.27) patch compartment of vehicle-pre-treated animals, while in DAMGO-pre-treated animals, methamphetamine also significantly increased prodynorphin mRNA expression in the patch compartment of both dorsolateral (t = 4.5; p = 0.001) and dorsomedial (t = 5.8; p = 0.0004) striatum. Furthermore, the effect of methamphetamine treatment on prodynorphin mRNA expression in the patch compartment was significantly greater in DAMGO-pre-treated animals than vehicle-pre-treated animals in the dorsomedial striatum (t = 3.7; p = 0.006), and trended towards significance in dorsolateral striatum (t = 2.9; p = 0.016). In the ventral aspects of striatum, two-way analysis of variance revealed a lack effect of DAMGO pre-treatment on prodynorphin mRNA expression in the patch compartment, and no interaction between DAMGO pre-treatment and methamphetamine treatment within either the ventrolateral or ventromedial sub-regions of striatum (Fig. 5d and e). However, there was a significant main effect of treatment for the patch compartment in the ventral aspects of striatum, with methamphetamine treatment significantly increasing prodynorphin RNA expression within the patch compartment of ventrolateral (t = 4.5; p = 0.0002) and ventromedial (t = 4.1; p = 0.0006) striatum.

Two-way analysis of variance of the effects of striatal mu opioid receptor activation on methamphetamine-induced prodynorphin mRNA expression in the matrix compartment of dorsomedial striatum revealed a significant effect of DAMGO pre-treatment, methamphetamine treatment and a significant pre-treatment × treatment interaction (Fig. 5c). Additional *post hoc* analysis revealed that methamphetamine treatment did not significantly increase prodynorphin mRNA expression in the dorsomedial matrix compartment of vehicle-pre-treated animals (t = 0.15; p = 0.88), but significantly increased prodynorphin mRNA expression in the dorsomedial matrix compartment of DAMGO-pre-treated animals (t = 4.8; p = 0.001), with a significantly greater effect of methamphetamine treatment on prodynorphin mRNA expression in

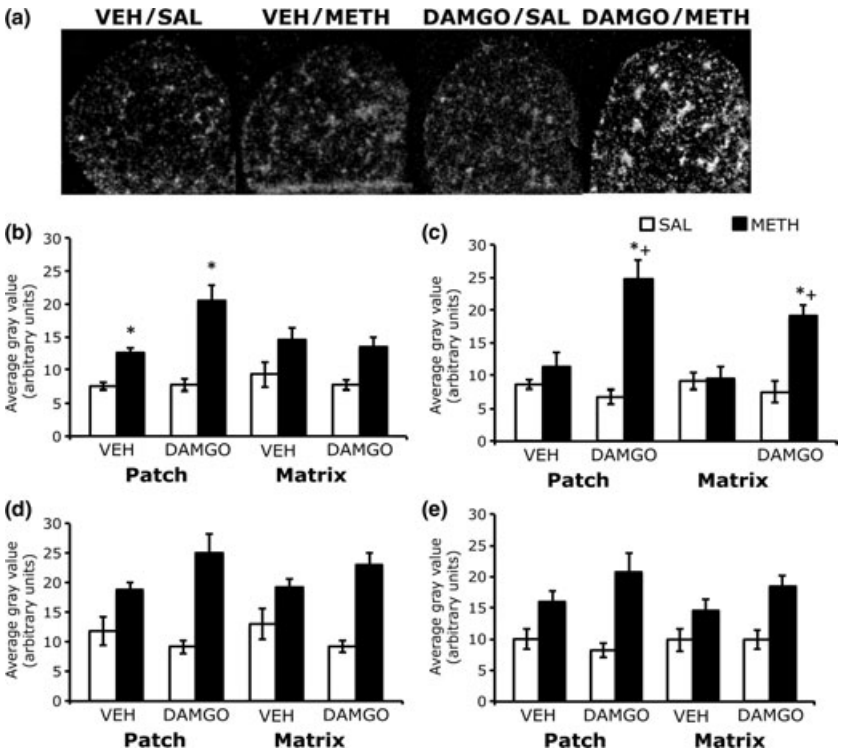

Fig. 5 Effects of DAMGO pre-treatment on methamphetamine-induced prodynorphin mRNA expression in the rostral striatum, 2 h post-treatment. *In situ* hybridization films (a) showing prodynorphin mRNA expression in the rostral striatum. Notice the slight increase in prodynorphin expression in vehicle-pre-treated, methamphetamine treated animals, and how the intensity of the prodynorphin mRNA expression is increased, particularly within the patches of DAMGO-pre-treated, methamphetamine-treated animals. Quantitative analysis of prodynorphin mRNA expression in the patch and matrix compartments of dorsolateral (b), dorsomedial (c), ventrolateral (d) and ventromedial (e) striatum, from rats intrastriatally infused with vehicle or DAMGO (1 μg/μL) 15 min prior to treatment with methamphetamine (0.5 mg/kg). Quantitative values are average gray values (arbitrary units, ± SEM, n = 5–8 animals). *Significantly different from vehicle-pre-treated control group, $p < 0.05$; $^{+}$Significantly different from vehicle-pre-treated methamphetamine-treated group, $p < 0.05$. There was a significant overall main effect of methamphetamine treatment in the patch and matrix compartments of ventromedial striatum and ventrolateral striatum.

DAMGO-pre-treated animals than vehicle-pre-treated animals (t = 3.7; p = 0.006). Two-way analysis of variance also revealed a lack of effect of DAMGO pre-treatment or a significant interaction between DAMGO pre-treatment and methamphetamine treatment in the dorsolateral, ventrolateral and ventromedial matrix compartments (Fig. 5b, d and e). However, there was a significant main effect of methamphetamine treatment in the matrix compartment of all three of these sub-regions, with methamphetamine treatment significantly increasing matrical prodynorphin mRNA expression in the dorsolateral (t = 3.9; p = 0.001), ventrolateral (t = 5.2; $p < 0.0001$) and ventromedial (t = 4.0; p = 0.0006) striatum. DAMGO pre-treatment did not significantly alter basal prodynorphin mRNA expression in the patch or matrix of any sub-region of striatum examined.

Effects of striatal mu opioid receptor activation on methamphetamine-induced stereotypy

Acute treatment with a low dose methamphetamine resulted in a mild to moderate level of stereotypy, which peaked during the first 45 min following treatment, and tapered off by 2 h post-treatment (Fig. 6a). Pre-treatment with DAMGO resulted in an increase stereotypic behavior in methamphetamine-treated animals, that also peaked at approximately 45 min post-methamphetamine treatment, followed by a gradual tapering-off by 2 h post-treatment (Fig. 6a). Two-way analysis of variance of the area under the curve values for stereotypy at the 45 min and 2 hh killing time points revealed that at both time points there were significant effects of DAMGO pre-treatment, methamphetamine treatment and a significant pre-treatment × treatment interaction. *Post hoc* analyses found that methamphetamine treatment significantly increased stereotypic behavior in vehicle-pre-treated animals, at 45 min (t = 7.5; $p < 0.0001$) and 2 h (t = 6.4, $p < 0.0001$) post-methamphetamine treatment, and in DAMGO-pre-treated animals, at 45 min (t = 30; $p < 0.0001$) and 2 h (t = 6.2; $p < 0.0001$) post-methamphetamine treatment. In addition, the degree of stereotypy induced by methamphetamine treatment was significantly greater in DAMGO-pre-treated versus vehicle-pre-treated animals, at both 45 min

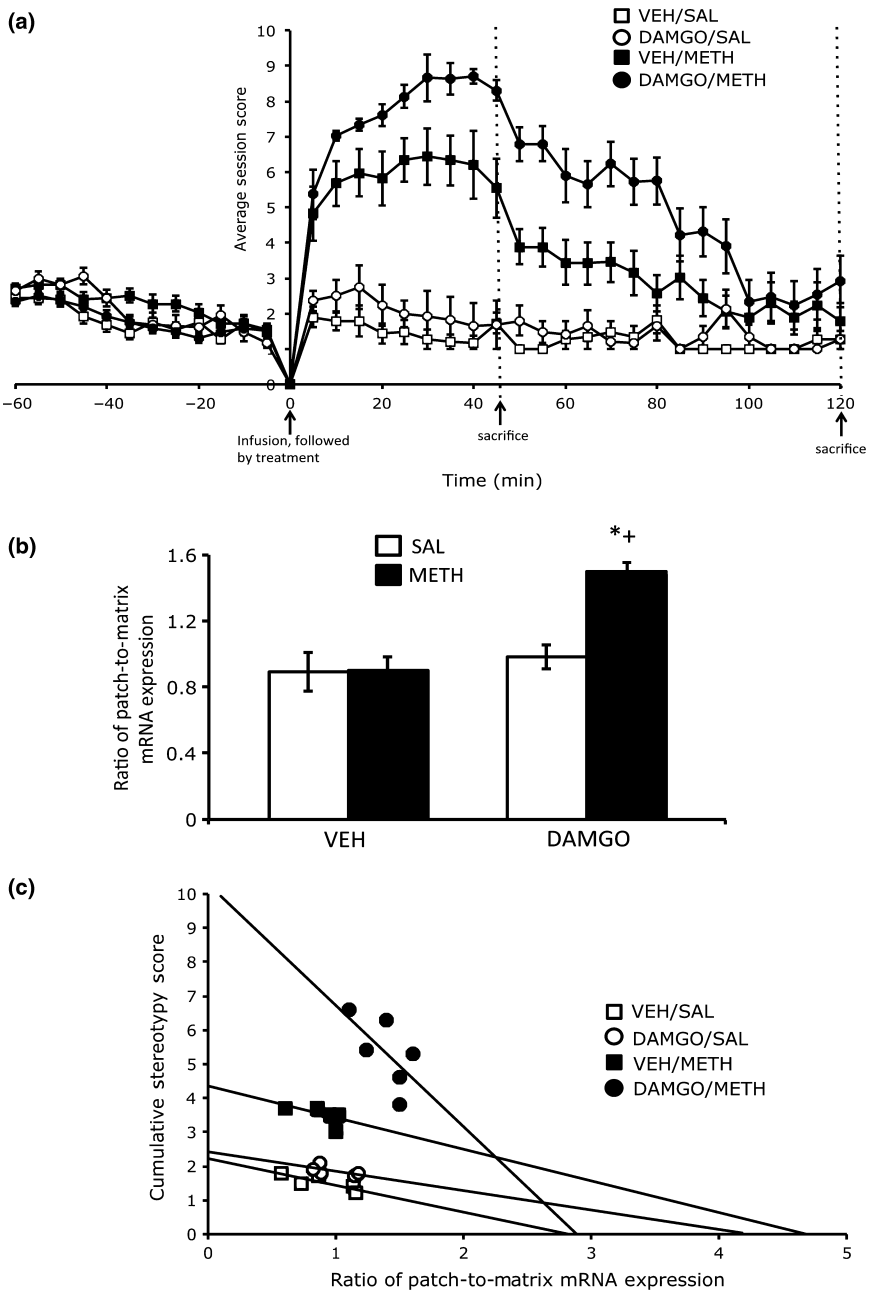

Fig. 6 Effects of intrastriatal infusion of DAMGO (1 μg/μL) and methamphetamine treatment (0.5 mg/kg) on stereotyped behavior (a). Values are expressed as the mean ± SEM. The two time points were combined into single graph for conciseness. Methamphetamine treatment significantly increased stereotypy at 45 min and 2 h post-treatment, and was enhanced by pre-treatment with DAMGO at both time points. The AUC values are as follows: at 45 min post-treatment, Vehicle/Saline = 61; Vehicle/methamphetamine (METH) = 203; DAMGO/Saline = 72; DAMGO/METH = 334 and 2 h post-treatment, Vehicle/Saline = 156; Vehicle/METH = 392; DAMGO/Saline = 187; DAMGO/METH = 610. Patch-enhanced gene expression in the dorsolateral striatum (b). Acute METH treatment did not significantly increase the ratio of patch-to-matrix prodynorphin mRNA expression in the dorsolateral striatum; however, pre-treatment with DAMGO resulted in a significant increase in the ratio of patch-to-matrix prodynorphin mRNA expression in this region. Correlation between cumulative stereotypy scores and the ratio of patch-to-matrix prodynorphin mRNA expression in the dorsolateral striatum (c). There was a significant negative correlation between the cumulative stereotypy scores and the ratio of patch-to-matrix prodynoprhin mRNA expression in DAMGO-pre-treated, methamphetamine-treated animals. *Significantly different from vehicle-pre-treated control group, $p < 0.05$; $^{+}$Significantly different from vehicle-pre-treated methamphetamine-treated group, $p < 0.05$.

($t = 6.9$; $p < 0.0001$) and 2 h ($t = 3.7$, $p = 0.001$) post-methamphetamine treatment.

Effects of DAMGO pre-treatment and methamphetamine treatment on the ratio of patch-to-matrix mRNA expression and correlation with stereotypy

Two-way analysis of variance of the ratio of patch-to-matrix prodynorphin mRNA expression, 2 h after treatment with a low dose of methamphetamine revealed that in the dorsolateral striatum, there were significant effects of DAMGO pre-treatment, methamphetamine treatment and a significant pre-treatment × treatment interaction. *Post hoc* analysis revealed that methamphetamine significantly increased the ratio of patch-to-matrix prodynorphin mRNA expression in the dorsolateral striatum of DAMGO-pre-treated ($t = 4.8$, $p = 0.0005$), but not vehicle-pre-treated ($t = 0.04$; $p = 0.97$) animals, as the effects of methamphetamine treatment on the ratio patch-to-matrix prodynorphin mRNA expression was significantly greater in DAMGO-pre-treated versus vehicle-pre-treated animals ($t = 5.5$; $p = 0.0001$; Fig. 6b). To examine the relationship between DAMGO pre-treatment, the relative expression of prodynorphin in the patch versus matrix compartments and stereotyped behavior in methamphetamine-treated animals, we determined if there was a correlation between the ratio of patch-to-matrix prodynorphin mRNA expression, for each treatment group, within each of the four sub-regions of striatum and the cumulative stereotypy scores for the entire 2 h behavioral session. At 2 h post-methamphetamine treatment, in the dorsolateral sub-region of striatum, there was a significant negative correlation between cumulative stereotypy scores and the ratio of patch-to-matrix prodynorphin mRNA expression in the dorsolateral striatum for DAMGO- ($r_s = -0.80$; $p = 0.03$) pre-treated, methamphetamine-treated animals, but not for vehicle-pre-treated methamphetamine-treated animals ($r_s = -0.89$; $p = 0.08$), vehicle-pre-treated saline-treated animals ($r_s = -0.90$; $p = 0.10$) or DAMGO-pre-treated, saline-treated animals ($r_s = -0.72$; $p = 0.23$; Fig. 6c). There was not a significant correlation between the ratio of patch-to-matrix prodynorphin mRNA expression and the cumulative stereotypy scores for the 2-h killing time point for any other sub-region of striatum examined (data not shown).

Two-way analysis of variance of the patch-to-matrix ratio of *zif/268* mRNA expression revealed that 45 min after methamphetamine treatment, the pattern of *zif/268* mRNA expression was patch-enhanced, but only in the dorsolateral striatum, as the ratio of patch-to-matrix *zif/268* mRNA expression was significantly greater in methamphetamine-treated versus saline-treated animals in this region ($t = 2.7$; $p = 0.014$). However, there was not a significant effect of DAMGO pre-treatment or a pre-treatment × treatment interaction, nor was there a significant correlation between patch-enhanced *zif/268* mRNA expression and cumulative stereotypy scores for the 45 min killing time point (data not shown). *Arc* and *c-fos* mRNA expression, 45 min following methamphetamine treatment and *zif/268* and *arc* mRNA expression, 2 h following methamphetamine treatment were not patch-enhanced in any sub-region of striatum, nor was there a significant effect of DAMGO pre-treatment or a significant pre-treatment × treatment interaction. In addition, there was not a significant correlation between the ratio of patch-to-matrix mRNA expression for *c-fos* or *arc* and the cumulative stereotypy scores at the 45-min killing time point or between the ratio of patch-to-matrix mRNA expression for *arc* or *zif/268* and cumulative stereotypy scores at the 2-h killing time point for any treatment group, in any striatal sub-region examined (data not shown).

Discussion

The goal of the current study was to determine whether activation of mu opioid receptors in the striatum would result in enhanced gene expression and stereotypic behavior when combined with a low dose of methamphetamine. Activation of striatal mu opioid receptors prior to treatment with a low dose of methamphetamine augmented *zif/268* mRNA expression in both the patch and matrix compartments of all four sub-regions of striatum, whereas methamphetamine-induced *c-fos* and *arc* mRNA expression in the patch and matrix compartments were unaltered by striatal mu opioid receptor activation in any sub-region of striatum. In addition, methamphetamine-induced prodynorphin expression was enhanced in the patch, but not matrix compartment of dorsolateral striatum by striatal mu opioid receptor activation and resulted in an increase the ratio of patch-to-matrix expression of prodynorphin mRNA in this region. Finally, mu opioid receptor activation significantly increased methamphetamine-induced stereotypy and induced a negative correlation between the ratio of patch-to-matrix prodynorphin mRNA expression in the dorsolateral striatum and the intensity of the stereotypic behavior. The current study provides additional evidence that striatal mu opioid receptor activation can differentially modulate methamphetamine-induced gene expression, as well as contribute to methamphetamine-induced stereotypy. These data also lend support to the notion that patch-enhanced dynorphin expression may serve as a homeostatic response to psychostimulant-induced overstimulation of the striatum.

Striatal mu opioid receptor activation and methamphetamine-induced immediate early gene mRNA expression

Activation of mu opioid receptors augmented methamphetamine-induced *zif/268* mRNA expression, with little effect on the expression of *arc* or *c-fos*. These data are similar to previous work from our laboratory where mu opioid receptor blockade attenuated methamphetamine-induced *zif/268*

mRNA expression, but had no effect on methamphetamine-induced *c-fos* mRNA expression (Horner and Keefe 2006; Horner *et al.* 2010). The differential regulation of methamphetamine-induced *zif/268* expression by mu opioid receptors could be the result of mu-mediated differences in calcium signaling and/or extracellular signal-regulated kinase (ERK) activity. Increases in nuclear calcium, which occur as a result of increased calcium influx at proximal synapses, may stimulate cyclic AMP response element (CRE)-mediated gene transcription, while increases in cytoplasmic calcium, which occur as a result of calcium influx at distal synapses, may stimulate serum response element (SRE)-mediated transcription (Ginty 1997; Hardingham *et al.* 1997). In addition, ERK-mediated Elk-1 activation (which targets SRE) takes place in the cytoplasm, while ERK-mediated CREB activation (which targets CRE) takes place in the nucleus (Sgambato *et al.* 1998). Interestingly, *zif/268* has four putative SREs and one or two CREs, *c-fos* has one SRE and three functional CREs and *arc* has a synaptic activity responsive element that comprises SRE, CRE and myocyte enhancer factor-2 binding sites (Christy *et al.* 1988; Sassone-Corsi *et al.* 1988; Kawashima *et al.* 2009). Activation of mu opioid receptors, which are found on the distal dendrites of medium spiny neurons in the striatum, has been shown to increase intracellular calcium, as well as ERK activity (Smith and Bolam 1990; Wang and Pickel 1998; Wang *et al.* 2000; Macey *et al.* 2006). Therefore, it is possible that activation of mu opioid receptors at distal synapses lead to increased calcium and Elk-1 activity in the cytoplasm, resulting in enhanced SRE-mediated *zif/268* transcription, while leaving nuclear calcium and CREB levels unaltered and thus CRE-mediated *c-fos* transcription unaffected. As the synaptic activity responsive element requires binding at both the SRE and CRE sites to initiate *arc* transcription, a lack of CREB (and possibly myocyte enhancer factor-2) activity may have prevented an enhancement of *arc* expression by mu opioid receptor activation.

Striatal mu opioid receptor activation and methamphetamine-induced gene expression in the matrix compartment

Interestingly, the selective enhancement of *zif/268* expression by mu opioid receptor activation was also apparent in matrix compartment, despite the relative paucity of mu opioid receptors in this sub-region, as compared with the patch compartment (Pert *et al.* 1976; Herkenham and Pert 1981; Tempel and Zukin 1987), but is in line with previous work from our laboratory where blockade of mu opioid receptors resulted in a diminution of *zif/268* mRNA expression in the matrix compartment (Horner and Keefe 2006; Horner *et al.* 2010). The mu opioid receptor-mediated changes in *zif/268* expression that originate within the patch compartment could be communicated to the matrix as a result of local interactions between the patch and matrix compartments. A portion of medium spiny neurons have dendritic arborizations that cross from one compartment into the other; thus dendrites from a portion of matrix neurons may cross over into the patch where they are influenced by DAMGO-induced changes in the patch compartment (Bolam *et al.* 1988; Walker *et al.* 1993). In addition, cholinergic interneurons have dendritic fields that extend across both compartments, and target the medium spiny neurons of the matrix with widespread axon collaterals (Bolam *et al.* 1988; Kawaguchi 1992; Tepper and Bolam 2004). However, if local circuit mechanisms were responsible for changes in the matrix compartment, then it is likely that the strongest effects of DAMGO infusion would be seen primarily around the site of injection. Alternatively, circuit-based mechanisms may be responsible for mu opioid receptor-mediated enhancement of methamphetamine-induced gene expression in the matrix. For example, activation of mu opioid receptors could reduce the activity of GABAergic medium spiny neurons in the patch compartment that send projections to the dopaminergic neurons of the substantia nigra pars compacta, relieving these neurons from inhibition and resulting in enhanced striatal dopamine release and increased striatal output (Gerfen 1984; Graybiel 1990; Gonzalez-Nicolini *et al.* 2003; Pereira *et al.* 2006). This increase in striatal output could lead to increased cortical activity via disinhibition of thalamocortical pathways, which may then increase corticostriatal input to the matrix, leading to enhanced gene expression in this region (Gerfen and Wilson 1996; Parthasarathy and Graybiel 1997; Sgambato *et al.* 1997). Interestingly, there appears to be enhanced cortical gene expression in DAMGO-pre-treated, methamphetamine-treated animals where *zif/268* mRNA expression was enhanced in the matrix (see Fig. 2a and 4a). However, it is important to note that this scenario does not explain how mu opioid receptor activation can simultaneously induce a patch-enhanced pattern of gene expression when combined with methamphetamine, or why this effect appears to be selective for certain genes. Clearly, additional studies are needed to further understand the potential contribution of mu opioid receptor activation to changes in circuit-level activity and the impact on methamphetamine-induced gene expression in the matrix compartment of striatum.

Striatal mu opioid receptor activation and methamphetamine-induced prodynorphin expression in the dorsolateral striatum

Treatment with a low dose of methamphetamine resulted in a homogenous pattern of prodynorphin mRNA expression in the dorsolateral striatum, as mRNA levels were significantly increased in the patch and matrix compartments, and to a similar degree. Activation of mu opioid receptors prior to treatment with methamphetamine significantly increased the level of prodynorphin expression in the patch, but not matrix compartment of dorsolateral striatum. Accordingly, striatal mu opioid receptor activation increased the ratio of patch-to-matrix prodynorphin mRNA expression in the dorsolateral

striatum, which, if we use the negative correlation between patch-enhanced prodynorphin expression and the intensity of stereotypy as a predictor, should result in a *decrease* in stereotypical behavior. However, this was not the case, as striatal mu opioid receptor activation *increased* methamphetamine-induced stereotypical behavior. One possible explanation for this incongruity is that striatal mu opioid receptor activation results in alterations in the release other neurotransmitters in the striatum. As mentioned above, reduced output from the patch compartment, as a result of mu opioid receptor activation, may disinhibit dopamine neurons in the substantia nigra pars compacta, resulting in enhanced striatal dopamine release and an exacerbation of methamphetamine-induced stereotypy (Gerfen 1984; Ujike *et al.* 1989; Graybiel 1990; Capper-Loup *et al.* 2002; Gonzalez-Nicolini *et al.* 2003; Pereira *et al.* 2006; Lan *et al.* 2009). Increased striatal dopamine release may then trigger a patch-enhanced pattern of prodynorphin expression, particularly in the dorsolateral striatum, possibly as a homeostatic response to dampen the overstimulation of striatal neurons, although the mechanism by which this may take place is unclear. Nevertheless, the dorsolateral striatum is though to play a role in repetitive behaviors and habit formation (Canales and Graybiel 2000; Yin and Knowlton 2006), and increased dynorphin expression in this region may serve to eventually reduce methamphetamine-induced stereotypy. A role for dynorphin as a homeostatic modulator in the striatum is supported by the observations that striatal prodynorphin expression is usually not evident until the peak of methamphetamine-induced stereotypy has passed and that kappa opioid receptor agonists can reduce stereotypic behavior and inhibit striatal dopamine release (Walker *et al.* 1987; Heidbreder *et al.* 1993; Toyoshi *et al.* 1996; You *et al.* 1999; Meshul and McGinty 2000; Ito *et al.* 2002; Margolis *et al.* 2003; Horner *et al.* 2010). However, it is important to note that psychostimulant-induced increases in *c-fos* and *arc* mRNA expression are dependent on dopamine D_1 receptor activation (Moratalla *et al.* 1996; Yamagata *et al.* 2000), and thus should have been affected if methamphetamine-induced dopamine release was augmented by DAMGO. Clearly, additional studies are needed to address the role of mu opioid receptor activation in methamphetamine-induced neurotransmitter release in the striatum, as well as the specific impact of these potential changes on methamphetamine-induced gene expression and behavior.

Striatal mu opioid receptor activation and methamphetamine-induced prodynorphin mRNA expression in the dorsomedial striatum

It is also interesting to note that mu opioid receptor activation significantly enhanced prodynorphin mRNA expression in both the patch and matrix compartments of dorsomedial striatum. Superimposed upon the topography cortical inputs to the patch and matrix compartments, is a medial-to-lateral topography of cortical inputs to the striatum as a whole, such that the medial aspects of the frontal cortex (e.g. the prelimbic cortex) project to the patch and matrix compartments of medial striatum, while the lateral aspects of frontal cortex (e.g. motor cortex) project to the patch and matrix compartments of lateral striatum (Gerfen 1989, 1992a). Thus, the medial striatum is also considered to be important for the transfer of limbic-related information through the basal ganglia. Of note is recent data that indicates psychostimulant-induced stereotypy may be the result of a functional imbalance between the medial prefrontal circuits that traverse the dorsomedial striatum and the sensorimotor circuits that traverse the dorsolateral striatum, as the psychostimulant-induced neurochemical and electrophysiological changes that occurred during stereotypy were observed in the medial prefrontal, but not sensorimotor circuits (Aliane *et al.* 2009). Thus, enhanced methamphetamine-induced prodynorphin expression in the dorsomedial striatum following DAMGO pre-treatment could reflect an imbalance in the medial prefrontal versus sensorimotor circuits through the striatum, and indicates that mu opioid receptor activation contributes to this imbalance. Interestingly, the methamphetamine-induced *c-fos* expression observed in the current study was restricted to the dorsomedial striatum, which also supports a functional imbalance between medial prefrontal versus sensorimotor circuits during stereotypy. However, methamphetamine-induced *c-fos* expression in the dorsomedial striatum was unaltered by DAMGO pre-treatment, which argues against a potential role for mu opioid receptor activation in the imbalance between medial prefrontal and sensorimotor circuits during stereotypy. Nevertheless, the relationship between methamphetamine-induced alterations in prodynophin and/or *c-fos* expression in the dorsomedial striatum and the functional imbalance between limbic and motor-based circuits through the basal ganglia and methamphetamine-induced stereotypy warrants further investigation.

Conclusions

Our findings demonstrate that striatal mu opioid receptor activation differentially contributes to methamphetamine-induced immediate early gene expression in the striatum. Furthermore, mu opioid receptor activation modulates *zif/268* expression in both the patch and matrix compartments of the striatum, suggesting that striatal mu opioid receptors may differentially modulate of intracellular signaling cascades and that compartmental cross-talk and/or circuit-level changes may allow for modification of methamphetamine-induced gene expression in both the patch and matrix compartments. Our findings also provide further evidence for a role of mu opioid receptors in the expression of methamphetamine-induced stereotypy, as well as methamphetamine-induced patch-enhanced pattern of prodynorphin mRNA expression. In addition, the current study confirms that striatal mu opioid receptors contribute to the negative relationship between

patch-enhanced pattern of prodynorphin mRNA expression in the dorsolateral striatum and the intensity of methamphetamine-induced stereotyped behavior. Together, these data indicate that the mu opioid receptor system contributes to the systemic changes in basal ganglia function and organismal behavior that occur as a result of methamphetamine treatment. The present data also lend support to the notion that patch-enhanced expression of prodynorphin of may be the response to methamphetamine-induced overstimulation of the striatum and stereotypy, rather than the source of methamphetamine-induced stereotypy.

Acknowledgements

The authors have no conflicts of interest to declare.

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