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Phasic-like stimulation of the medial forebrain bundle augments striatal gene expression despite methamphetamine-induced partial dopamine denervation

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Abstract

Methamphetamine-induced partial dopamine depletions are associated with impaired basal ganglia function, including decreased *preprotachykinin* mRNA expression and impaired transcriptional activation of activity-regulated, cytoskeleton-associated (*Arc*) gene in striatum. Recent work implicates deficits in phasic dopamine signaling as a potential mechanism linking methamphetamine-induced dopamine loss to impaired basal ganglia function. The present study thus sought to establish a causal link between phasic dopamine transmission and altered basal ganglia function by determining whether the deficits in striatal neuron gene expression could be restored by increasing phasic dopamine release. Three weeks after pretreatment with saline or a neurotoxic regimen of methamphetamine, rats underwent phasic- or tonic-like stimulation of ascending dopamine neurons. Striatal gene expression was examined using *in situ* hybridization histochemistry. Phasic-like, but not tonic-like, stimulation induced immediate-early genes *Arc* and *zif268* in both groups, despite the partial striatal dopamine denervation in methamphetamine-pretreated rats, with the *Arc* expression occurring in presumed striatonigral efferent neurons. Phasic-like stimulation also restored *preprotachykinin* mRNA expression. These results suggest that disruption of phasic dopamine signaling likely underlies methamphetamine-induced impairments in basal ganglia function, and that restoring phasic dopamine signaling may be a viable approach to manage long-term consequences of methamphetamine-induced dopamine loss on basal ganglia functions.

Keywords

methamphetamine; striatum; dopamine; *Arc*; *preprotachykinin*; *zif268*

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Introduction

Methamphetamine (METH) is an addictive psychostimulant that is neurotoxic to dopamine (DA) neurons. Markers of DA innervation in both the dorsal and ventral striatum are reduced in brains of chronic METH abusers (Wilson *et al*. 1996), and individuals with a history of hospitalization associated with METH use are more likely to develop Parkinsonism (Callaghan *et al*. 2012). Thus, it is likely that some proportion of individuals who abuse METH will experience a period of partial DA loss as a consequence of their METH abuse prior to the development of Parkinsonism. Prior studies have suggested a relation between METH-induced DA loss and cognitive impairment in individuals with a history of METH abuse, as reduction of dopamine transporter (DAT) binding in the caudate-putamen correlates with motor and cognitive impairments in METH users (Volkow *et al*. 2001). Furthermore, METH use is associated with cognitive decline, as executive functions, learning, and memory, which are dependent on intact striatal circuitry (Brown *et al*. 1997; Packard and Knowlton 2002), are impaired in METH users (Scott *et al*. 2007; Dean *et al*. 2013 , but see Hart *et al*. 2012). Similarly, in rodents, exposure to METH causes significant striatal DA denervation (Hotchkiss and Gibb 1980; Ricaurte *et al*. 1980; Ricaurte *et al*. 1982). Associated with this depletion are learning impairments, including impaired recognition of novel stimuli (Schroder *et al*. 2003; Marshall *et al*. 2007; Herring *et al*. 2008; O'Dell *et al*. 2011), impaired motor-sequence learning (Chapman *et al*. 2001; Daberkow *et al*. 2005), and altered reversal learning (Izquierdo *et al*. 2010; Pastuzyn *et al*. 2012). Despite the association between METH-induced DA denervation and learning impairments, the molecular mechanisms linking these phenomena are not fully understood.

Approximately 95% of striatal neurons are GABAergic medium spiny neurons (MSNs), which are found in two roughly equal subtypes. Striatonigral (“direct pathway”) MSNs express D1 DA receptors, as well as the neuropeptides substance P (and its *preprotachykinin* (*ppt*) precursor) and dynorphin (Gerfen *et al*. 1990; Surmeier *et al*. 1996). Striatopallidal neurons (“indirect pathway”) express D2 DA receptors, as well as the neuropeptide enkephalin (and its *preproenkephalin* (*ppe*) precursor) (Gerfen *et al*. 1990; Le Moine *et al*. 1990; Surmeier *et al*. 1996). Partial striatal DA loss induced by either METH or 6-hydroxydopamine (6-OHDA) results in a reduction in basal *ppt* expression in striatum, but no change in *ppe* expression (Nisenbaum *et al*. 1996; Chapman *et al*. 2001; Johnson-Davis *et al*. 2002). Furthermore, METH-induced neurotoxicity is associated with loss of *Arc* (activity-regulated, cytoskeleton-associated gene) transcription in response to behavioral activation (Daberkow *et al*. 2008; Barker-Haliski *et al*. 2012a), and only the impairment in the numbers of striatonigral neurons with *Arc* mRNA in the cytoplasm correlates significantly with the degree of METH-induced striatal DA loss (Barker-Haliski *et al*. 2012a). Taken together, these data suggest that partial DA loss, such as that induced by METH, affects striatal efferent neuron function and that this dysfunction may predominantly affect striatonigral neurons.

METH-induced neurotoxicity selectively impairs phasic-like DA signaling (Howard *et al*. 2011), which is thought to preferentially affect striatonigral neurons (Chergui *et al*. 1997; Gonon 1997; Onn *et al*. 2000). METH-induced partial DA depletion is associated with diminished amplitude of DA signals evoked using phasic-like stimulation of the DA neurons ascending through the medial forebrain bundle (MFB)(Howard *et al*. 2011), as well as the amplitude of endogenous, spontaneously occurring phasic DA transients (CD Howard, *et al*., submitted). On the other hand, tonic DA levels, which are thought to be sufficient to activate D2 receptors expressed by striatopallidal neurons (Richfield *et al*. 1989; Dreyer *et al*. 2010), are not disrupted following METH pretreatment, as assessed by microdialysis (Cass and Manning 1999). Interestingly, enhancing phasic-like, but not tonic-like, DA signaling via electrical stimulation of DA neurons results in increased *zif268* expression in D1 DA

receptor-containing striatonigral neurons (Chergui *et al*. 1997). Therefore, METH-induced dysfunction in direct pathway MSNs may be due to impairments in phasic, but not tonic, DA signaling.

If reduced phasic DA signaling is related to the gene expression deficits observed following METH-induced partial DA denervation, then augmenting phasic DA signaling should restore normal gene expression in the partially DA denervated striatum. We tested this hypothesis by pretreating rats with a neurotoxic regimen of METH and then, three weeks later, stimulating DA neurons ascending through the MFB in either a phasic- or tonic-like pattern. We then assessed striatal expression of *ppt* and *ppe* and the immediate-early genes (IEGs) *Arc* and *zif268*. We show that phasic-like stimulation increases IEG expression in both METH- and saline-pretreated rats and that the increase in *Arc* mRNA expression is preferentially in direct pathway neurons. Furthermore, the phasic-like stimulation restores *ppt* expression in METH-pretreated rats without altering *ppe* expression. These findings suggest that restoring phasic DA signaling may ameliorate basal ganglia dysfunction arising consequent to partial DA depletion, such as that induced by METH.

Materials and Methods

Animals

Adult male Sprague-Dawley rats (n=30, 240–350 g at time of pretreatment) were purchased from Harlan (Indianapolis, IN, USA) and housed in a light- and temperature-controlled vivarium. Access to food and water was provided *ad libitum*. All procedures conformed to the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of Illinois State University.

Drugs

(±)-Methamphetamine hydrochloride was provided by the National Institute on Drug Abuse (Rockville, MD, USA). METH doses were calculated as free base. All other chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA).

METH pretreatment

The “binge” neurotoxic METH regimen was conducted as previously described (Howard *et al*. 2011). Briefly, animals were housed in plastic tub cages (50 cm length × 40 cm width × 20 cm height: four rats/cage). METH was dissolved in 0.9% saline and all injections were made subcutaneously. Four injections of either METH (7.5 mg/kg) or saline were administered at 2-h intervals. Temperature was monitored rectally using a Thermalert TH-5 (Physitemp, Clifton, NJ, USA) prior to, immediately after the first injection, and every hour thereafter, continuing two hours after the final injection of METH. Health was assessed at least every hour, and if rats showed signs of overheating, they were placed in a separate tub on ice for ~10 min.

Electrical stimulation and in vivo voltammetry

Three weeks after METH or saline pretreatment, animals to be stimulated were anesthetized with urethane (1.5 g/kg, i.p.) and placed in a stereotaxic apparatus (David Kopf Instruments, Tajunga, CA, USA). Four holes were drilled into the skull to allow for lowering of the stimulating electrode, two carbon fiber microelectrodes (CFM), and a Ag/AgCl reference electrode. The twisted, bipolar stimulating electrode (Plastics One, Roanoke, VA, USA) was placed dorsal to the MFB (−4.6 AP; +1.4 ML; −7.0 DV; Paxinos and Watson 1986) and was incrementally lowered until a robust DA signal was recorded in the striatum at the CFMs placed in the dorsomedial (DM) and dorsolateral (DL) striatum (+1.2 AP; +1.0 and +4.0 ML, respectively; −4.5 DV; Paxinos and Watson 1986) at 6° angles to allow side by side

ipsilateral placement. Changes in DA concentration were recorded using fast-scan cyclic voltammetry (FSCV), where a triangular waveform (−0.4 V to 1.3 V and back at 400 V/s) was applied to the tip of the CFM every 100 ms (Cahill *et al*. 1996) using an EI400 bipotentiostat (Ensman Instruments, Bloomington, IN, USA) that was computer-controlled using TH-1 software (ESA, Chelmsford, MA, USA). Current recorded at each CFM was converted to concentration using *in vitro* calibration immediately following experiments (Logman *et al*. 2000). The purpose of recording with FSCV at a CFM in this experiment was to identify DA neurons ascending through the MFB for stimulation with phasic- and tonic-like pulse trains, and to ensure optimal stimulating electrode placement. Non-stimulated control rats were anesthetized with urethane, but did not undergo surgical manipulations or any stimulation.

Following optimization of the stimulating electrode placement, no stimulation was given for 1 h to reduce optimization- and handling-induced striatal gene expression (Daberkow *et al*. 2007). The experimental stimulation protocol was then begun. Electrical stimulation was optically isolated (NL 800, Neurolog, Medical Systems, Great Neck, NY, USA) and synchronized with FSCV recordings. The stimulation protocol was chosen based on previous work noting enhanced expression of mRNA for the IEG *zif268* following phasic-like ("burst"), but not tonic-like ("regular"), stimulation (Chergui *et al*. 1997). Stimulation was comprised of constant-current, biphasic pulses (2 ms and 300 μA each phase) and was delivered in either a phasic-like (5 pulses at 30 Hz repeated every 1 s for 60 s; n=5 METH-pretreated; n=5 saline-pretreated) or tonic-like (300 pulses at 5 Hz; n=5 METH-pretreated; n=5 saline-pretreated) pattern. Stimulation trains were 60 s in duration and were repeated a total of 15 times, with 2 min separating each stimulation train, for a total stimulation session time of 45 min. Immediately following stimulation sessions, animals were euthanized and brains were rapidly extracted. Non-stimulated control rats were sacrificed 180 min after being anesthetized with urethane (n=5 METH-pretreated; n=5 saline-pretreated). Brains were flash frozen in 2-methylbutane (EMD Millipore, Billerica, MA, USA) on dry ice and stored at −80°C.

Dopamine transporter (DAT) autoradiography

To determine the extent of METH-induced DA depletions, frozen brains were sectioned (12-μm coronal sections) using a cryostat (Cryocut 1800; Leica, Wetzlar, Germany). Sections were thaw-mounted onto Superfrost Plus (VWR, Aurora, CO, USA) slides. Slides were stored at −20°C until needed. DA transporter (DAT) autoradiography was performed as detailed previously by others and us (Boja *et al*. 1992; O'Dell *et al*. 2011; Pastuzyn *et al*. 2012). Briefly, slides were incubated in buffer containing fluoxetine to block radioligand binding to the serotonin transporter in striatum. Slides then were incubated in the continued presence of fluoxetine with [^{125}I]RTI-55 (PerkinElmer, Waltham, MA, USA). Slides were then rinsed, dried under a stream of warm air, and exposed to film (Kodak Biomax MR; Eastman Kodak, Rochester, NY, USA) for 24 h before being developed.

Radioactive in situ hybridization

To assess striatal IEG expression, frozen slides containing striatal sections were post-fixed and delipidated as previously described (Ganguly and Keefe 2001). As detailed previously, detection of *ppt* (Chapman *et al*. 2001; Johnson-Davis *et al*. 2002; Horner *et al*. 2005), *ppe* (Ganguly and Keefe 2000; Chapman *et al*. 2001; Ganguly and Keefe 2001; Daberkow *et al*. 2007), *zif268* (Keefe and Ganguly 1998; Keefe and Adams 1998), and *Arc* (Daberkow *et al*. 2007; Daberkow *et al*. 2008; Barker-Haliski *et al*. 2012a) mRNAs was accomplished using ribonucleotide probes. Antisense ribonucleotide probes were transcribed from linearized plasmids using ^{35}S-UTP (PerkinElmer) and SP6 (*ppe* and *ppt*) or T7 (*zif268* and *Arc*) RNA polymerases (Roche, Indianapolis, IN, USA). The radioactive *in situ* hybridization was

performed as previously described (Ganguly and Keefe 2001) with slight modifications to final washing procedures. The last four washes on the second day were either at 55°C (*Arc* and *ppt*) or room temperature (*ppe* and *zif268*). Slides were dipped in ddH_2O, air-dried, and exposed to film. Exposure times were: *ppt*, *zif268*, and *Arc*, two weeks; *ppe*, three days.

Fluorescent in situ hybridization

To assess pathway-specific expression of *Arc* mRNA, slides were post-fixed and delipidated as for radioactive *in situ* hybridization above. As previously described (Daberkow *et al*. 2007; Barker-Haliski *et al*. 2012a), expression of *Arc* and *ppe* mRNAs in striatum was determined by performing double-label fluorescent *in situ* hybridization (FISH) using probes directed against *Arc* and *ppe* mRNAs. *Arc* and *ppe* antisense ribonucleotide probes were synthesized using digoxigenin-UTP (DIG-UTP) and fluorescein-UTP (FITC-UTP) with T7 and SP6 RNA polymerases using DIG or FITC labeling kits (Roche), respectively. Slides were hybridized and detected as previously described (Daberkow *et al*. 2007; Daberkow *et al*. 2008; Barker-Haliski *et al*. 2012b), except that a 1:50,000 solution of SYTOX Green (Molecular Probes; Life Technologies, Grand Island, NY, USA) was used as a nuclear stain instead of DAPI. As controls, a set of slides was run in parallel either without ribonucleotide probes or without antibodies. Lack of signal on these slides was taken as evidence of the specificity of the *in situ* hybridization histochemical labeling.

Image analysis

Films from DAT autoradiography and radioactive *in situ* hybridization histochemistry were developed, and images were digitized using a video camera (CCD72S; Dage-MTI, Michigan City, IN, USA) and fiber optic light box. The intensity of the light was adjusted so that it fell within the linear range of the camera, as determined by a photographic step tablet (Eastman Kodak Co.). Densitometric analysis of the digitized images was then accomplished using NIH ImageJ software, yielding average gray values in DM and DL striatum for both hemispheres. Two rostral (+1.6 mm from Bregma) and two middle striatal sections (+0.7 mm from Bregma) were analyzed per rat. For each hemisphere, the average gray value of the corpus callosum was subtracted from the average gray value of DM and DL striatum to correct for background staining. Decreases in DAT in METH-pretreated rats were calculated as a percent of DAT levels in saline-pretreated rats.

FISH images were collected using a Leica DM4000B automated upright microscope (63X oil immersion objective) connected to a Leica EL6000 external light source with a mercury metal halide bulb and a Leica DFC300 FX digital color camera. Surveyor computer software (Objective Imaging Ltd.; Cambridge, UK) was used to control the automated stage, perform multichannel scanning, and capture the fluorescent images. A 2 × 2 montage (0.38 mm^2) was captured in the area of striatum with the most *Arc* expression, as identified from the film autoradiograms of *Arc* mRNA expression. In the case of rats with no apparent stimulation-induced *Arc* mRNA expression on the film autoradiograms, montages of FISH staining were captured in DL striatum, as DL striatum was the location where the majority of rats stimulated in a phasic-like manner showed *Arc* expression. The total numbers of *Arc*-positive/*ppe*-negative (i.e., presumed striatonigral neurons) and *Arc*-positive/*ppe*-positive (i.e., striatopallidal neurons) neurons in each image were counted by an experimenter blinded to the treatment groups. Furthermore, the average signal intensity of *Arc* expression in each individual neuronal population (striatonigral and striatopallidal) was measured in ImageJ by first individually outlining all *Arc*-positive/*ppe*-positive neurons in an image and measuring signal intensity in those cells. Then, the *Arc*-positive/*ppe*-negative neurons in the image were outlined and the average *Arc* signal intensity of those neurons was measured.

Statistical analysis

Rectal temperatures were compared using repeated measures MANOVA with pretreatment as a factor and time as a repeated measure. *Post hoc* one-way ANOVAs with Tukey-Kramer HSD tests were used to further interrogate the significant pretreatment × time interaction for the body-temperature data. For radioactive *in situ* hybridization and DAT autoradiography, average gray values were compared between pretreatment (METH or saline) and stimulation (no stimulation, tonic, or phasic) groups by two-way ANOVA. For FISH, the numbers of *Arc*-positive/*ppe*-negative and *Arc*-positive/*ppe*-positive cells were compared between pretreatment and stimulation groups by two-way ANOVA. The relative amount of *Arc* signal in the striatal neuron populations was also examined by calculating a "Difference Score", which was the average gray value of the *Arc*-positive/*ppe*-negative population minus the average gray value of the *Arc*-positive/*ppe*-positive population for each animal. This "Difference Score" was compared between pretreatment and stimulation groups by two-way ANOVA. *Post hoc* Tukey-Kramer HSD tests were performed when ANOVA revealed significant interactions or main effects. Statistical tests were performed using JMP (v. 9.0) or SAS (v. 9.3) software.

Results

METH pretreatment and DAT autoradiography

A treatment × time repeated measures MANOVA revealed that the METH "binge" pretreatment paradigm differentially altered body temperature across METH- and saline-treated rats (Fig. 1a; significant effect of time, $F_{(7,30)}=4.45$, $p=0.0032$; significant effect of treatment $F_{(1,30)}=53.43$, $p<0.0001$; significant time × treatment interaction, $F_{(7,30)}=35.20$, $p<0.0001$). *Post hoc* analysis revealed that METH-treated animals were significantly hyperthermic relative to saline-treated animals two hours after METH injection and every hour thereafter ($p<0.001$ at 120–420 min after METH). METH pretreatment resulted in significant decreases in striatal DAT three to five weeks later, as assessed by [^{125}I]RTI-55 autoradiography (Fig. 1b–c). A two-way ANOVA revealed a main effect of pretreatment (DM striatum, $F_{(1,24)}=17.1$, $p<0.001$; DL striatum, $F_{(1,24)}=20.3$, $p=0.0001$), but no significant main effect of stimulation and no significant pretreatment × stimulation interaction ($p>0.05$).

Effect of phasic- and tonic-like stimulation of the MFB on Arc expression

Stimulation of the MFB resulted in changes in striatal *Arc* expression in both saline- and METH-pretreated rats (Fig. 2a). A two-way ANOVA (pretreatment × stimulation) for *Arc* mRNA expression in DM striatum revealed a main effect of stimulation (Fig. 2b; $F_{(2,24)}=5.29$, $p<0.05$), but no significant effect of pretreatment ($p>0.05$) and no significant stimulation × pretreatment interaction ($p>0.05$). *Post hoc* analysis of the main effect of stimulation revealed that phasic-like stimulation increased *Arc* mRNA expression in the DM striatum both relative to non-stimulated controls ($p=0.03$) and rats receiving tonic-like stimulation ($p<0.05$). However, *Arc* mRNA expression in rats receiving tonic-like stimulation was not different from the non-stimulated control group ($p>0.05$).

Similarly, in DL striatum, there was a main effect of stimulation (Fig. 2b; $F_{(2,24)}=7.24$, $p<0.01$), but no main effect of pretreatment ($p>0.05$) and no significant stimulation × pretreatment interaction ($p>0.05$). *Post hoc* analysis again revealed that phasic-like stimulation increased *Arc* mRNA expression relative to that seen in non-stimulated controls ($p<0.01$) and rats receiving tonic-like stimulation ($p=0.03$). As in DM striatum, there was no significant difference in *Arc* mRNA expression in rats receiving tonic-like stimulation relative to non-stimulated controls ($p>0.05$). Thus, regardless of whether rats had partial DA

loss induced by METH pretreatment, phasic-like activation increased the expression of *Arc* mRNA in DM and DL striatum.

Effect of phasic- and tonic-like stimulation of the MFB on zif268 expression

Stimulation of the MFB resulted in changes in striatal *zif268* expression in both saline- and METH-pretreated rats that were similar to those observed for *Arc* (Fig. 2c). A two-way ANOVA (pretreatment × stimulation) in DM striatum revealed a main effect of stimulation (Fig. 2d; $F_{(2,24)}$=9.46, p<0.001), but no effect of pretreatment (p>0.05) and no significant interaction (p>0.05). *Post hoc* analysis of the main effect of stimulation revealed that *zif268* expression was significantly greater in rats receiving phasic-like stimulation relative to both rats receiving tonic-like stimulation (p<0.001) or no stimulation (p<0.001). The expression of *zif268*, however, was not different between rats receiving tonic-like stimulation and rats receiving no stimulation (p>0.05).

Likewise, in DL striatum, there was a significant main effect of stimulation (Fig. 2d; $F_{(2,24)}$=8.12, p<0.01), but no main effect of pretreatment (p>0.05) and no significant interaction (p>0.05). Again, *post hoc* analysis revealed that *zif268* expression in rats receiving phasic-like stimulation was significantly greater than that in rats receiving tonic-like stimulation (p<0.01) or no stimulation (p<0.01). The expression of *zif268* was not significantly different between rats receiving tonic-like stimulation and non-stimulated controls (p>0.05). Thus, as was the case for *Arc*, phasic- but not tonic-like stimulation of the MFB increased *zif268* expression in both METH- and saline-pretreated rats.

Effects of phasic- and tonic-like stimulation of the MFB on ppt expression

We and others (Nisenbaum *et al*. 1996; Chapman *et al*. 2001; Johnson-Davis *et al*. 2002) have previously reported that partial DA loss, such as that induced by METH pretreatment, results in a long-term decrease in *ppt* expression. Consistent with this prior work, in the present study, METH-pretreatment was associated with a decrease in *ppt* mRNA expression (Fig. 3a, c, d). Two-way ANOVA of *ppt* mRNA expression in DM striatum revealed a main effect of pretreatment (Fig. 3c; $F_{(1,24)}$=4.87, p<0.05), with *ppt* expression being lower overall in METH-pretreated rats. The ANOVA also revealed a main effect of stimulation (Fig. 3b; $F_{(2,24)}$=4.6, p<0.05), but no significant interaction (p>0.05). *Post hoc* analysis of the main effect of stimulation revealed that phasic-like stimulation significantly elevated *ppt* mRNA expression in DM striatum relative to rats receiving tonic-like stimulation of MFB (p<0.02).

In DL striatum, there was a trend towards an effect of pretreatment (Fig. 3d; $F_{(1,24)}$=3.34; p=0.08) and a main effect of stimulation (Fig. 3b; $F_{(2,24)}$=3.67, p<0.05), but no significant interaction (p>0.05). *Post hoc* analysis revealed strong trends indicating that phasic-like stimulation increased *ppt* expression in DL striatum relative to both that seen in rats receiving tonic-like stimulation (p=0.057) and control rats that did not receive stimulation (p=0.084). As with the other genes, *ppt* mRNA expression was not different between rats receiving tonic-like stimulation vs. no stimulation controls (p=0.98).

Effects of phasic- and tonic-like stimulation of the MFB on ppe expression

Neither METH pretreatment nor stimulation of the MFB in either a phasic-like or tonic-like pattern resulted in any significant changes in striatal *ppe* mRNA expression in saline- or METH-pretreated rats (Fig. 3e–f). Thus, in both DM and DL striatum, there were no significant main effects of pretreatment or stimulation and no significant interactions (all p>0.05).

Effects of phasic- and tonic-like stimulation of the MFB on Arc expression in subpopulations of striatal neurons

Stimulation of the MFB did not cause any changes in the numbers of *ppe*-negative (presumed striatonigral) and *ppe*-positive (striatopallidal) neurons positive for *Arc* mRNA expression ($p>0.05$; Fig. 4a–b). However, it was clear during blinded image analysis that the relative amount of signal for *Arc* mRNA was different between striatonigral and striatopallidal neurons (Fig. 4c–f). Thus, we measured the relative average gray value of *Arc* expression in the two efferent neuron populations separately and calculated a "Difference Score" (see Methods) reflecting the difference in the average gray value of the *Arc* signal in *ppe*-negative vs. *ppe*-positive neurons. Two-way ANOVA of this "Difference Score" revealed a significant main effect of stimulation ($F_{(2,24)}=9.3$, $p=0.001$), but no main effect of pretreatment ($p>0.05$) and no significant interaction ($p>0.05$). *Post hoc* analysis of the main effect of stimulation revealed that greater *Arc* mRNA expression in *ppe*-negative neurons was induced by phasic-like stimulation relative to either tonic-like stimulation ($p<0.001$) or no stimulation ($p<0.02$).

Discussion

Consistent with prior reports in the literature, the present results show that partial striatal DA loss, such as that induced by a neurotoxic regimen of METH, is associated with decreased *ppt* mRNA expression in the striatum (Nisenbaum *et al.* 1996; Chapman *et al.* 2001; Johnson-Davis *et al.* 2002). Furthermore, the present results confirm prior work (Chergui *et al.* 1997) that phasic-like stimulation of the MFB increases *zif268* expression in striatum. Additionally, the present work extends these previous studies by showing that phasic-like stimulation also increases *Arc* and *ppt* mRNA expression in striatonigral neurons and, importantly, that this effect of phasic-like stimulation is effective in driving gene expression in presumed striatonigral efferent neurons even in the setting of partial DA loss induced by METH. Thus, the present data reinforce the idea that phasic-like DA neuron activity appears to selectively affect the function of striatonigral efferent neurons and suggest that sufficient circuitry remains in rats with partial striatal DA loss to restore striatal function by enhancing phasic DA neurotransmission

The present data suggest that augmentation of phasic-like DA neurotransmission in animals with partial striatal DA loss may generally restore the ability of residual DA neurons to regulate striatonigral efferent neuron gene expression. As noted above, partial DA loss, such as that induced by 6-OHDA or a neurotoxic regimen of METH, is associated with an impairment of phasic-like, but not tonic-like, DA signals (Bergstrom and Garris 2003; Howard *et al.* 2011). It has been proposed, based on electrophysiological (Onn *et al.* 2000) and computer modeling (Dreyer *et al.* 2010) studies, that D1 DA receptors, and thus striatonigral efferent neurons (Gerfen and Surmeier 2011), are most sensitive to the higher levels of extracellular DA resulting from phasic DA activity. Alternatively, it has been suggested that D2 DA receptors, which are selectively expressed by striatopallidal efferent neurons, are largely saturated by DA under tonic extracellular DA levels and thus are largely insensitive to phasic increases in extracellular DA levels (Dreyer *et al.* 2010). Taken together, these data suggest that the decrease in phasic DA neurotransmission associated with partial DA loss selectively impairs D1 receptor activation and, therefore, normal gene expression in striatonigral neurons. Our present results further suggest that augmenting phasic-like DA neurotransmission can restore the degree of D1 dopamine receptor activation and, thus, striatonigral neuron function.

Consistent with this model, expression of *ppe* mRNA in D2 DA receptor-expressing striatopallidal neurons was not altered either by METH-induced neurotoxicity or by phasic- or tonic-like stimulation of the MFB in this study. Previous studies from our lab and others

that found a decrease in *ppt* expression in the setting of partial striatal DA loss also reported no change in *ppe* mRNA expression (Nisenbaum *et al.* 1996; Chapman *et al.* 2001; Johnson-Davis *et al.* 2002). Interestingly, expression of *ppe* mRNA in striatopallidal neurons only changes (increases) in the setting of extensive (~80–90%) denervation of the striatum (Gerfen *et al.* 1991; Nisenbaum *et al.* 1996), and this same degree of striatal DA denervation is necessary before a decrease in tonic, extracellular levels of DA occurs (Abercrombie *et al.* 1990; Castaneda *et al.* 1990). Given that METH neurotoxicity resulted in an ~25% striatal DA denervation (Fig. 1c), METH-induced neurotoxicity is not associated with changes in tonic extracellular levels of DA (Robinson *et al.* 1990; Cass and Manning 1999). It is therefore not surprising that the METH-pretreated rats in this study, as in previous reports, did not have changes in *ppe* expression.

One confound of the current study is non-selective stimulation of axons in the MFB. The MFB is highly heterogeneous and contains non-dopaminergic neurons that project to both striatum and the cortex (Nieuwenhuys *et al.* 1982). Additionally, the striatum receives glutamatergic afferents from various cortical areas (McGeorge and Faull 1989; Ramanathan *et al.* 2002), and electrical stimulation of the cortex augments striatal IEG expression in monkey (Parthasarathy and Graybiel 1997) and rat (Fu and Beckstead 1992; Liste *et al.* 1995). Therefore, it is possible that the gene expression measured here was partially induced through a non-dopaminergic pathway, potentially relayed through the cortex. However, electrical stimulation of MFB released DA in the striatum as measured by FSCV (data not shown), the stimulating electrode was optimized within the MFB to elicit this DA release, and previous work has demonstrated that the D1 antagonist SCH23390 impairs expression of *zif268* (*NGFI-A*) caused by MFB stimulation (Chergui *et al.* 1997). Furthermore, as is apparent in Fig. 2 and Fig. 3, cortical activation of *Arc* and *zif268* expression is notable in animals that received phasic- or tonic-like stimulation, whereas only animals receiving phasic-like stimulation showed increased striatal IEG expression. We noted no correlation between gene expression in striatum and that in the cortex directly overlying striatum in either stimulation group (data not shown), suggesting that the cortical and striatal gene expression are not linked. Thus, these data suggest that the striatal gene expression is likely induced by electrical stimulation of DA neurons ascending through the MFB, although we cannot definitively rule out a contribution of other circuits to the effects at present.

Somewhat analogous to METH-induced neurotoxicity, Parkinson's disease (PD) is characterized in part by loss of striatal DA nerve terminals (Hornykiewicz and Kish 1987) and dysfunction in striatal gene products (Nisbet *et al.* 1995). Importantly, cognitive impairments have been recognized in PD patients during the preclinical stage (Abbruzzese *et al.* 2009). While the pathology underlying these cognitive impairments is not understood, theoretical models indicate that deficits in D1 receptor signaling may play a role (Frank *et al.* 2004; Guthrie *et al.* 2009; Wiecki and Frank 2010). It is thus informative that decreases in indices of D1 DA receptor-expressing striatonigral neuron function, including transcriptional activation of IEGs essential for consolidation of long-term memories (Barker-Haliski *et al.* 2012a), and deficits in basal ganglia-mediated learning and memory functions are apparent in rats with partial DA depletions (less than 80%), such as those induced by METH (Chapman *et al.* 2001; Daberkow *et al.* 2005; Daberkow *et al.* 2008; Son *et al.* 2011; Pastuzyn *et al.* 2012). Taken together, these data suggest that deficits in phasic DA signaling and downstream striatonigral gene expression alterations may be involved in the cognitive disabilities apparent in both the setting of METH-induced neurotoxicity and the preclinical stages of PD. The present data further suggest that approaches to augment residual phasic DA signaling in the context of partial DA denervation or to replicate such signaling in the setting of more extensive DA denervation may prove fruitful in managing cognitive deficits associated with deficits in DA signaling, such as those observed in individuals with a history of METH abuse or PD.

Our findings demonstrate that augmenting phasic DA signaling in the partially DA denervated striatum enhances striatal gene expression to the same extent as in the intact striatum. Deficits in striatonigral neuron gene expression induced by large DA-depleting brain lesions are reversed with administration of levodopa (L-DOPA) (Zeng *et al.* 1995; Westin *et al.* 2001). Given that L-DOPA increases vesicular content of DA (Pothos *et al.* 1996) and electrically evoked DA release in intact rats (Garris *et al.* 1994; Rodriguez *et al.* 2007), it seems likely that it will augment phasic DA signaling in the context of partial striatal DA loss. Whether the post-synaptic consequences of partial DA denervation can be reversed with L-DOPA treatment or administration of other agents that enhance phasic DA signaling, such as amphetamine (Ramsson *et al.* 2011; Daberkow *et al.* 2013), which is used as a cognitive enhancer in treating attention-deficit hyperactivity disorder and drug addiction (Brady *et al.* 2011; Steiner and Van Waes 2013), is currently being examined.

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Text abbreviations

Arc	activity-regulated cytoskeleton-associated gene
CFM	carbon fiber microelectrode
DA	dopamine
DAT	dopamine transporter
DIG	digoxigenin
DL	dorsolateral
DM	dorsomedial
FISH	fluorescent *in situ* hybridization
FITC	fluorescein
FSCV	fast-scan cyclic voltammetry
IEG	immediate-early gene
MFB	medial forebrain bundle
METH	methamphetamine
ppe	*preproenkephalin*
ppt	*preprotachykinin*

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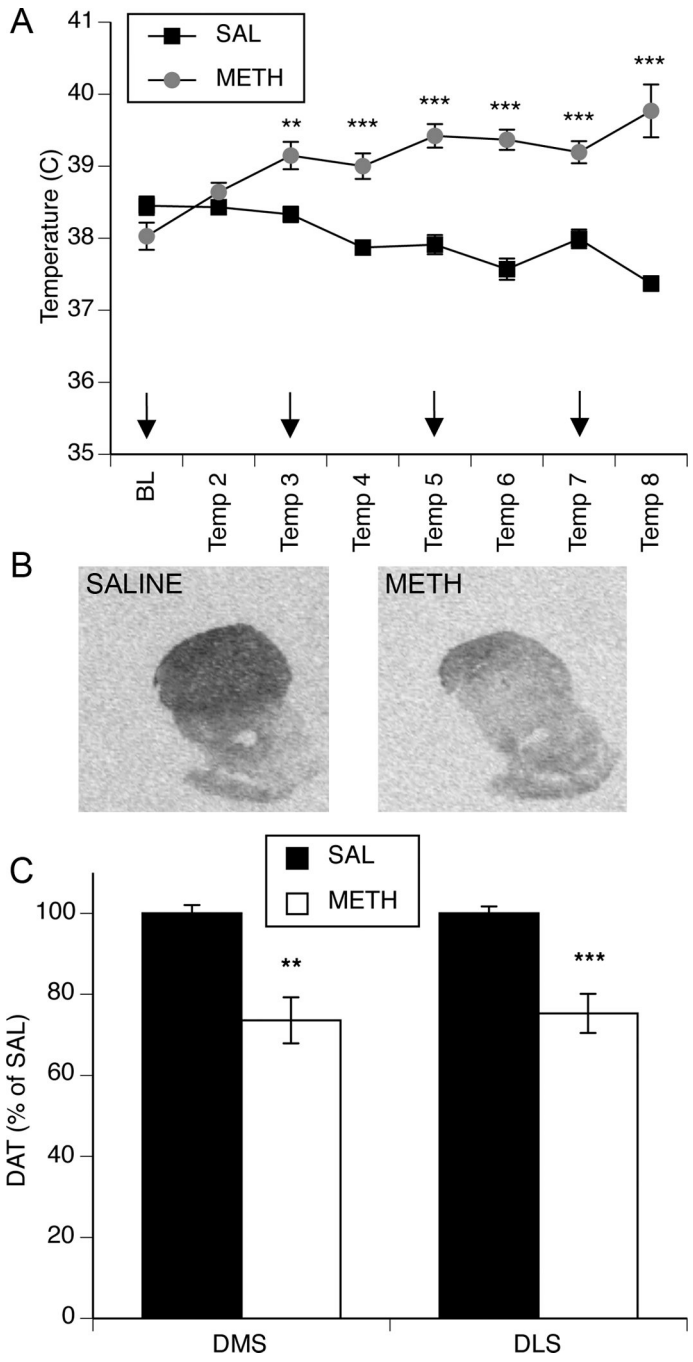

Fig. 1. Effect of methamphetamine pretreatment on body temperature and dopamine innervation in the dorsal striatum

(A) METH treatment resulted in significantly increased body temperatures relative saline-treated controls. Arrows indicate time of METH injections. Data are average rectal temperatures (°C; mean±SEM, n=15 for saline-treated and n=15 for METH-treated). *Significantly different from saline-treated rats at the same time point. BL, baseline; **p<0.001, ***p<0.0001. **(B)** Representative images of [^{125}I]RTI-55 striatal DAT binding in a saline- and METH-pretreated rat 3–5 weeks after METH pretreatment. **(C)** METH pretreatment resulted in partial dopamine denervation in both the dorsomedial (DMS) and dorsolateral (DLS) striatum 3–5 weeks after METH pretreatment as assessed by

[^{125}I]RTI-55 binding to dopamine transporters. Data are presented as percent of saline-pretreated values (±SEM, n=15 for saline-pretreated and n=15 for METH-pretreated). *Significant effect of METH pretreatment. **p<0.001, ***p<0.0001.

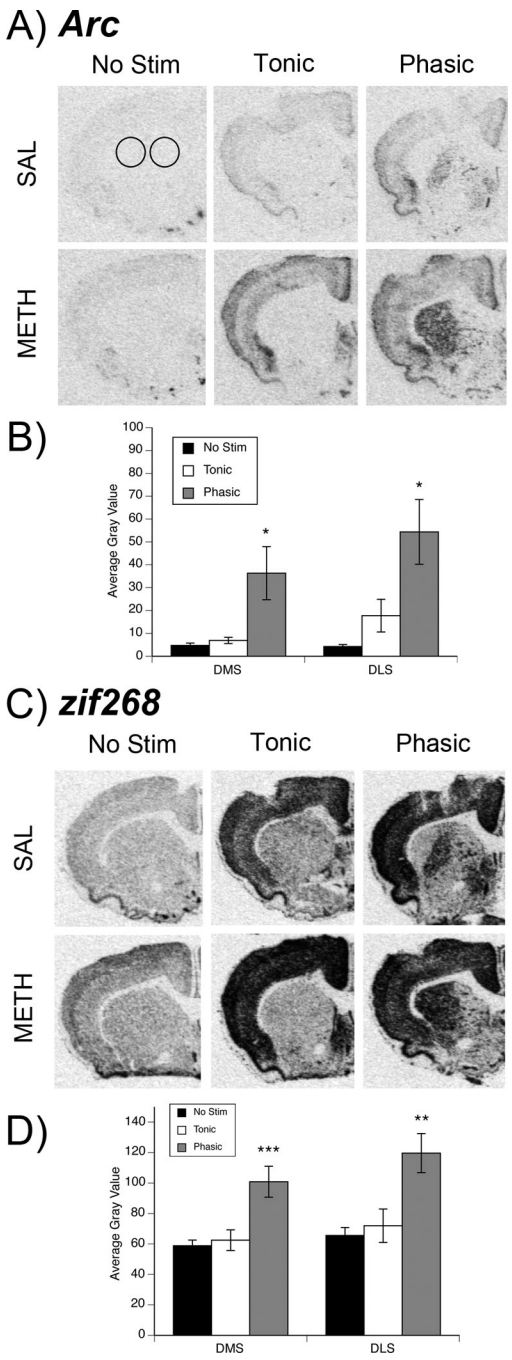

Fig. 2. Effect of MFB stimulation on expression of *Arc* and *zif268* in striatum of METH- and saline-pretreated rats

(A, C) Representative images of striatal *Arc* (A) or *zif268* (C) mRNA expression in saline- (top row) and METH- (bottom row) pretreated rats. Circles on the *Arc*/saline-pretreated/no stimulation image represent the regions of interest (ROIs) measured in both dorsomedial (DMS) and dorsolateral (DLS) striatum for all genes in this study. **(B, D)** Quantification of *Arc* (B) and *zif268* (D) expression in stimulation groups collapsed across pretreatment group. Data are average gray values (arbitrary units; mean±SEM; n=10 for each stimulation group per subregion, per gene product). *Significantly different from both no stimulation

(No Stim) and Tonic groups, $p<0.05$. **Significantly different from both No Stim and Tonic groups, **$p<0.01$. ***Significantly different from both No Stim and Tonic groups, $p<0.001$.

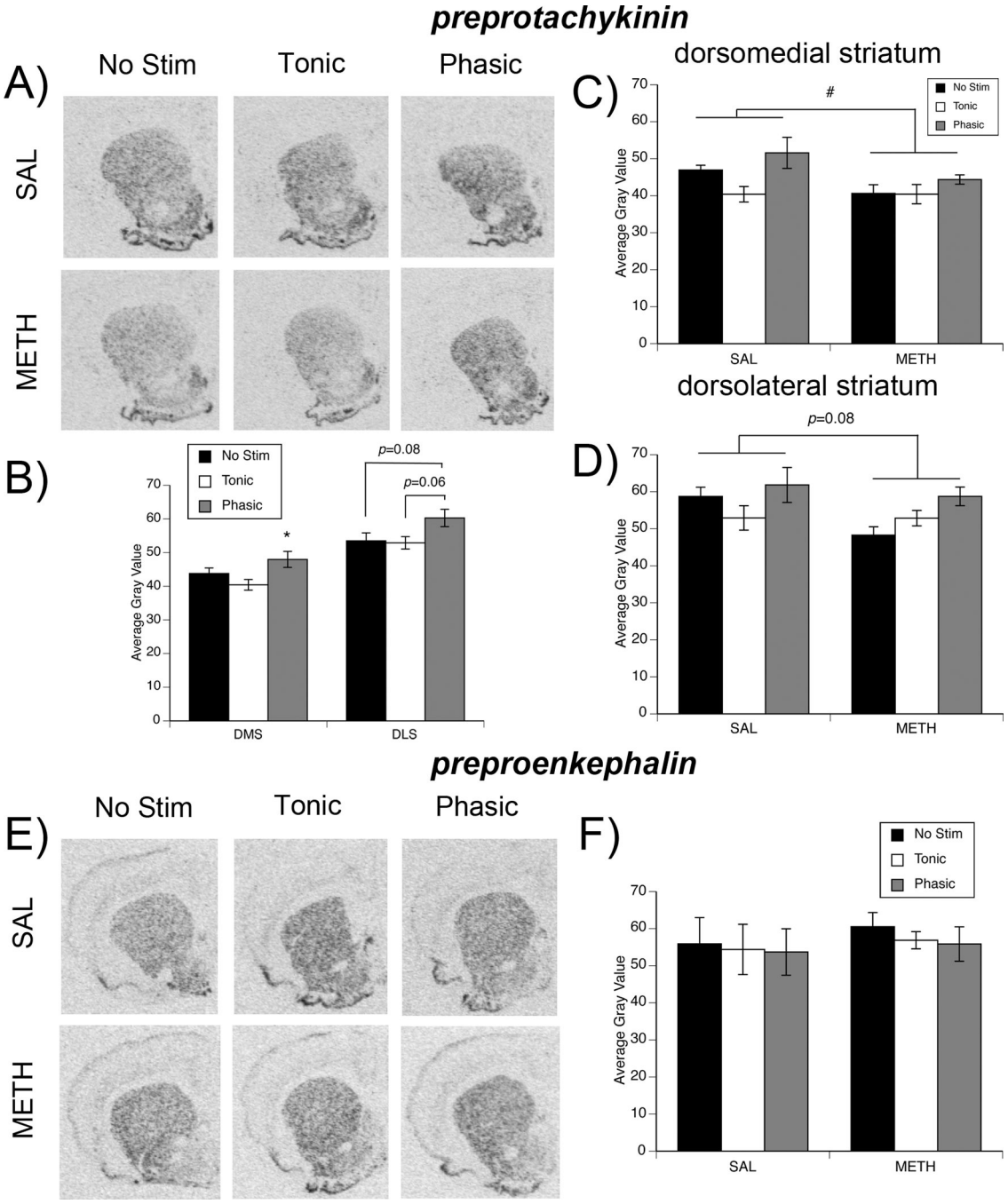

Fig. 3. Effect of MFB stimulation on expression of *preprotachykinin* and *preproenkephalin* in striatum of METH- and saline-pretreated rats

(A) Representative images of striatal *ppt* mRNA expression in saline- (top row) and METH- (bottom row) pretreated rats. **(B)** Quantification of *ppt* expression in stimulation groups collapsed across pretreatment groups. Data are average gray values (arbitrary units; mean ±SEM, n=10 for each stimulation group per subregion). *Significantly different from Tonic group, p<0.05. **(C, D)** Quantification of *ppt* expression separated by pretreatment group to demonstrate the effect of METH pretreatment on *ppt* expression. Data are average gray values (arbitrary units; mean±SEM, n=5 for saline- and METH-pretreated animals per stimulation group for both DMS and DLS). #Main effect of pretreatment, p <0.05. **(E)**

Representative images of striatal *ppe* mRNA expression in saline- (top row) and METH- (bottom row) pretreated rats. **(F)** Quantification of *ppe* expression in dorsomedial striatum. Data are average gray values (arbitrary units; mean±SEM, n=5 for saline- and METH-pretreated animals per stimulation group). Data from dorsolateral striatum are not shown, as there was no subregion difference.

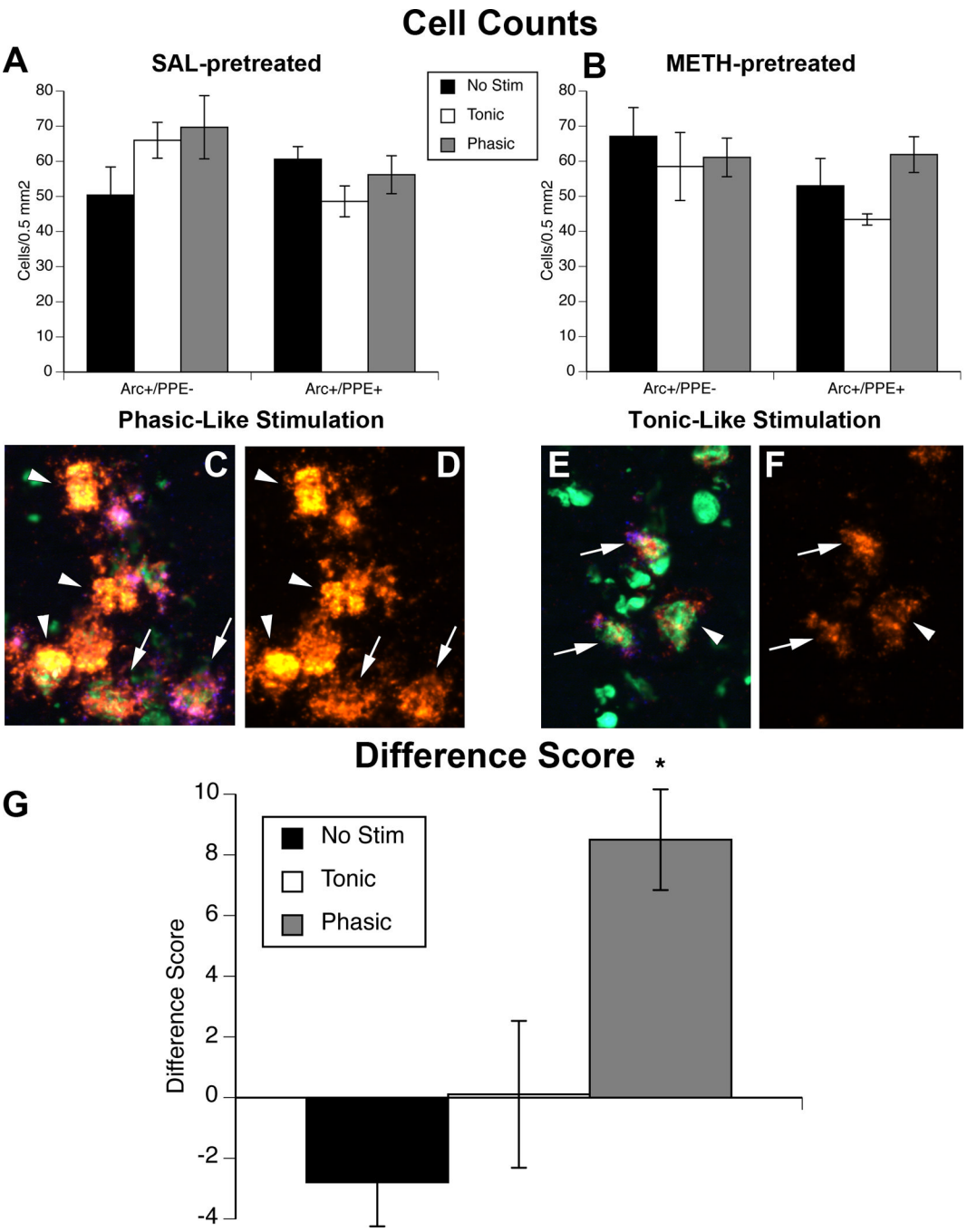

Fig. 4. Phasic-like stimulation of dopamine neurons increases *Arc* expression in striatonigral neurons

(A–B) Numbers of *Arc*-positive/*ppe*-negative and *Arc*-positive/*ppe*-positive cells/0.5 mm^2 in the striatum of A) saline (SAL)- and B) METH-pretreated rats receiving no stimulation (No Stim) or tonic-like or phasic-like stimulation of the MFB. **(C–F)** Representative fluorescent *in situ* hybridization images of *Arc* mRNA expression in presumed striatonigral neurons (*Arc*-positive/*ppe*-negative; arrowheads) and striatopallidal neurons (*Arc*-positive/*ppe*-positive; arrows) in rats that received phasic-like (C–D) or tonic-like (E–F) stimulation. *Arc* is orange, *ppe* is blue, SYTOX nuclear stain is green. Images in C and E show all three channels, while images in D and F show only the *Arc* channel. Scale bar=10 μm. **(G)** Graph

showing the "Difference Score" collapsed across pretreatment ($p>0.05$), defined as the difference between the average gray value of *Arc*-positive/*ppe*-negative and *Arc*-positive/*ppe*-positive neurons. *Significantly different from Tonic and No Stim groups, $p<0.05$, $p<0.001$, respectively.