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METHAMPHETAMINE-INDUCED NEUROTOXICITY DISRUPTS PHARMACOLOGICALLY EVOKED DOPAMINE TRANSIENTS IN THE DORSOMEDIAL AND DORSOLATERAL STRIATUM

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Abstract

Phasic dopamine (DA) signaling, during which burst firing by dopamine neurons generates short-lived elevations in extracellular DA in terminal fields called DA transients, is implicated in reinforcement learning. Disrupted phasic DA signaling is proposed to link DA depletions and cognitive-behavioral impairment in methamphetamine (METH)-induced neurotoxicity. Here we further investigated this disruption by assessing effects of METH pretreatment on DA transients elicited by a drug cocktail of raclopride, a D2 DA receptor antagonist, and nomifensine, an inhibitor of the dopamine transporter (DAT). One advantage of this approach is that pharmacological activation provides a large, high-quality data set of transients elicited by endogenous burst firing of DA neurons for analysis of regional differences and neurotoxicity. These pharmacologically evoked DA transients were measured in the dorsomedial (DM) and dorsolateral (DL) striatum of urethane-anesthetized rats by fast-scan cyclic voltammetry. Electrically evoked DA levels were also recorded to quantify DA release and uptake, and DAT binding was determined by autoradiography to index DA denervation. Pharmacologically evoked DA transients in intact animals exhibited a greater amplitude and frequency and shorter duration in the DM compared to the DL striatum, despite similar pre- and post-drug assessments of DA release and uptake in both sub-regions as determined from the electrically evoked DA signals. METH pretreatment reduced transient activity. The most prominent effect of METH pretreatment on transients across striatal sub-region was decreased amplitude, which mirrored decreased DAT binding and was accompanied by decreased DA release. Overall, these results identify marked intrastriatal differences in the activity of DA transients that appear independent of presynaptic mechanisms for DA release and uptake and further support disrupted phasic DA signaling mediated by decreased DA release in rats with METH-induced neurotoxicity.

Keywords

methamphetamine; dopamine; transient; release; uptake; voltammetry

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INTRODUCTION

Human and animal studies have established that methamphetamine (METH)-induced neurotoxicity is associated with striatal dopamine (DA) depletions and behavioral-cognitive impairments (Simon et al. 2000; Volkow et al. 2001; Daberkow et al. 2005; Izquierdo et al. 2010; O'Dell et al. 2011; Pastuzyn et al. 2012; for reviews, see Nordahl et al. 2003; Scott et al. 2007; Marshall and O'Dell 2012). Diminished DA signaling has thus been investigated as a possible link. DA neurons fire in two modes, tonic and phasic (Hyland et al. 2002; Grace et al. 2007). Tonic or basal firing, in combination with intracellular actions of terminal heteroreceptors, produces a low, steady-state concentration of extracellular DA called “tone” (Venton et al. 2003; Borland and Michael 2004), whereas phasic or burst firing generates short-lived elevations on top of tone called “transients” (Somers et al. 2009; Owesson-White et al. 2012). Emerging evidence suggests that METH-induced neurotoxicity leads to a selective disruption of phasic, but not tonic, DA signaling (Cass and Manning 1999; Howard et al. 2011; Loewinger et al. 2012; Howard et al. 2013a). Similar differential changes in DA signaling have been demonstrated in two other conditions of striatal DA depletion: experimental parkinsonism (Abercrombie et al. 1990; Robinson et al. 1994; Garriss et al. 1997; Bergstrom and Garriss 2003; Bergstrom et al. 2011) and traumatic brain injury (Wagner et al. 2005; Wagner et al. 2009a; Wagner et al. 2009b).

The postulate that a disruption in phasic DA signaling links DA depletions and behavioral-cognitive impairment in METH-induced neurotoxicity (Howard et al. 2011) is compelling, because of the importance of this communication mode in reward prediction error during reinforcement learning (Schultz 1998; Schultz 2007; Morita et al. 2012). Moreover, neurocomputational models predict cognitive deficits in Parkinson's disease with diminished phasic DA signaling (Frank et al. 2004; Wiecki and Frank 2010), and functional magnetic resonance imaging demonstrates reduced striatal signals representing reward prediction error in patients with Parkinson's disease (Schonberg et al. 2010). However, with the exception of one study that evaluated naturally occurring DA transients (Howard et al. 2013a), all other studies in animal models of striatal DA depletion assessed electrically evoked phasic-like DA signals (see above). While these phasic-like signals can precisely mirror the extracellular dynamics of DA transients with judicious selection of stimulus parameters (Robinson et al. 2001; Cheer et al. 2005; Howard et al. 2011), there is evidence suggesting distinct regulation of the two (Budygin et al. 2001; Cheer et al. 2004; Wightman et al. 2007; Robinson et al. 2009; Brown et al. 2011). There is also evidence supporting intrastriatal differences in the control of phasic DA signaling (Zhang et al. 2009a; Zhang et al. 2009b; Brown et al. 2011; Willuhn et al. 2012). It is thus important to establish the effects of partial DA loss on non-electrically evoked phasic DA signals in striatal sub-regions.

Here we investigated the effects of METH-induced neurotoxicity on DA transients elicited by raclopride (RAC), a D2 DA receptor antagonist, and nomifensine (NOM), an inhibitor of the dopamine transporter (DAT). Administration of a cocktail of these two drug classes to anesthetized animals has previously been shown to robustly enhance burst firing of DA neurons (Shi et al. 2000; Shi et al. 2004) and DA transients (Venton and Wightman 2007; Park et al. 2010; Park et al. 2011). Thus, this pharmacological approach permits studying

DA transients elicited by endogenous burst firing in the anesthetized preparation, in which phasic DA signaling is suppressed (Kelland et al. 1990; Koulchitsky et al. 2012), and also supports simultaneous microsensor recording in two brain regions to enhance intrastriatal comparisons of the effects of DA depletions (Bergstrom and Garris 2003; Bergstrom et al. 2011; Howard et al. 2011). We show here using fast-scan cyclic voltammetry (FSCV) in urethane-anesthetized rats that the activity of pharmacologically evoked DA transients was greater in the dorsomedial (DM) compared to the dorsolateral (DL) striatum in intact (i.e., saline-pretreated) animals, and was reduced in both striatal sub-regions by METH pretreatment.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (250–450 g), purchased from Harlan Industries (Indianapolis, IN, USA), were used for all experiments. Animals were housed under conditions of controlled lighting and temperature, and food and water were provided *ad libitum*. All care was in accordance with the *Guide for the Care and Use of Laboratory Animals* (2011; National Academies Press, Washington, DC, USA) and was approved by the Institutional Animal Care and Use Committee of Illinois State University.

METH pretreatment

A binge dosing regimen was used to induce METH neurotoxicity (Howard et al. 2011; Howard et al. 2013a; Howard et al. 2013b). This regimen reliably mimics aspects of the long-term neurotoxic effects of METH observed in humans, including loss of markers for DA and serotonin neurons and microglia activation (Marshall and O'Dell 2012). Briefly, animals were placed in plastic housing tubs (50 cm length × 40 cm width × 20 cm height; 4 rats/tub). Saline (0.9 %) or (±)-METH•HCl (7.5 mg/kg; dissolved in 0.9% saline; calculated as free base) was injected s.c. every two hours until a total of four injections were administered. Core body temperature was monitored rectally (Thermalert TH-5 thermometer, Physitemp, Clifton, NJ, USA) every hour during injections and for two additional hours after injections.

Surgery

Rats were anesthetized with urethane (1.5 g/kg i.p.) and immobilized in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA) (Howard et al. 2011; Howard et al. 2013b). Core temperature was maintained with Deltaphase Isothermal Pads (Braintree Scientific, Braintree, MA, USA). Holes were drilled through the skull in order to position recording, stimulating, and reference electrodes using coordinates from a rat brain atlas (Paxinos and Watson 1986). Bregma and dura were used as reference points, and all coordinates are in mm. Initially, the stimulating electrode was positioned just dorsal to the medial forebrain bundle (AP -4.6, ML +1.4, DV -7.0), carbon-fiber microelectrodes were lowered ipsilaterally into the DM and DL striatum (AP +0.4, ML +1.8 and +3.8, respectively, DV -4.5), and the reference electrode was placed in the contralateral superficial cortex. The stimulating electrode and carbon-fiber microelectrodes were then incrementally

lowered to record robust levels of electrically evoked DA. After optimization, the position of these electrodes was not changed throughout the duration of the experiment.

Voltammetry

Extracellular DA was monitored by FSCV at a carbon-fiber microelectrode (Howard et al. 2011; Howard et al. 2013a; Howard et al. 2013b). Carbon-fiber microelectrodes were fabricated by aspirating a single carbon fiber (3.5- μ m radius; Cytec Engineering Materials, West Patterson, NJ, USA) into a borosilicate glass capillary and pulling to a taper (PE-2 Micropipette Puller, Narishige, Japan). The carbon fiber was trimmed to a length of ~100 μ m extending beyond the glass insulation. FSCV was performed by an EI400 bipotentiostat (Ensmann Instruments, Bloomington, IN, USA) under the control of TH-1 software (ESA, Chelmsford, MA, USA). The potential of the carbon-fiber microelectrode was linearly scanned from -0.4 V to +1.3 V (versus Ag/AgCl) and back every 100 ms at a rate of 400 V/s. Current due to the oxidation of DA was measured at approximately +0.6 V (versus Ag/AgCl) and converted to concentration based on post-calibration (Wu et al. 2001a). DA was identified by the background-subtracted cyclic voltammogram (Michael et al. 1998). For electrically evoked DA signals, 10 scans, beginning 5 s before application of the stimulus train, were averaged for background subtraction. For analysis of DA transients, background subtraction was performed for each scan based on a moving average of 10 scans, typically collected beginning 1.5 s before that scan. The reference electrode (Ag/AgCl) was a chloridized silver wire.

Electrical stimulation

A twisted bipolar stimulating electrode (MS 303/2, Plastics One, Roanoke, VA, USA) was used to activate DA axons ascending through the medial forebrain bundle. Electrical stimulation was computer controlled and synchronized to FSCV by the TH-1 software. Trains consisting of biphasic stimulus pulses (300 μ A and 2 ms each phase) were delivered at a frequency of 60 Hz. Optimization trains were 2 s (120 pulses) in duration, whereas trains applied during pre- and post-drug recording were 1 s (60 pulses) in duration. Trains applied during pre- and post-drug recording were used to elicit DA signals for determining DA release and uptake. Although these electrically evoked DA signals are phasic-like, pulse number and intensity are greater than those typically used to model DA transients (Robinson et al. 2001; Cheer et al. 2005; Howard et al. 2011). Thus, these electrically evoked DA signals were not considered DA transients in the present study. Stimulus pulses were constant-current and optically isolated (NL 800 Neurolog, Medical Systems, Great Neck, NY, USA).

Data analysis

Phasic DA events were determined from non-electrically evoked recordings by MINI ANALYSIS peak-finding software (Synaptosoft, Inc., Decatur, GA, USA) (Howard et al. 2013a). Signals identified by FSCV as DA and greater than 5-times the root-mean-square of background noise were considered phasic events (Howard et al. 2013a) and were assessed for amplitude, duration (i.e., width at half-peak height), and inter-transient interval (ITI). Phasic events with a duration $\leq$ 2 s were considered DA transients, and those with a duration $>$ 2 s were considered DA "surges". This cutoff was selected, because most spontaneously

active DA transients recorded in the absence and presence of a DAT inhibitor have a duration shorter than 2 s (Robinson and Wightman 2004; Wightman et al. 2007; Aragona et al. 2008). Noise artifacts were manually removed.

Electrically evoked recordings of DA were analyzed for maximal concentration ($[DA]_{\max}$) and parameters describing the presynaptic mechanisms of DA release and uptake (Howard et al. 2011; Daberkow et al. 2013; Howard et al. 2013a). Two equations were used; both considered the rate of change of extracellular DA elicited by electrical stimulation as a balance between the opposing processes of DA release and uptake (Wightman et al. 1988). The first equation assumed DA uptake to follow Michaelis-Menten kinetics:

$$d[DA]/dt = [DA]_p * f - V_{\max} / (K_m / [DA] + 1) \quad \text{Eq. 1}$$

where DA release is described by the concentration of DA released per stimulus pulse or $[DA]_p$, $V_{\max}$ is the maximal rate of DA uptake, K_m is inversely related to the affinity of DA for DAT, and f is the frequency of stimulation. Eq. 1 was used to determine the pre-drug values for $[DA]_p$ and $V_{\max}$ described in Table 1. These values were compared to DA depletion as assessed by DAT binding, because these values have been previously shown to decrease with DA innervation in METH-induced neurotoxicity (Howard et al. 2011). K_m was assumed to be 0.2 μM for this analysis (Wu et al. 2001b). The second equation assumed DA uptake to follow first-order kinetics:

$$d[DA]/dt = [DA]_p * f - k * [DA] \quad \text{Eq. 2}$$

where k is the first-order rate constant. Eq. 2 was used to determine the pre- and post-drug values for $[DA]_p$ and k described in Figure 2 and Table 2, which assessed the effects of the RAC-NOM drug cocktail on presynaptic DA function. An assumption of first-order kinetics was used to characterize DA uptake in this analysis to avoid complications due to RAC changing $V_{\max}$ and NOM changing K_m (Wu et al. 2001a; Wu et al. 2002). A simplex minimization algorithm was used for fitting simulated curves calculated by both equations to experimental data (Wu et al. 2001b).

DAT Binding

Upon completion of FSCV recording, animals were sacrificed, brains were rapidly removed and frozen in 2-methyl butane on dry ice, and tissue samples were stored at -80°C until processing for DAT binding. Brains were sectioned coronally (12 μm) using a cryostat (Cryocut 1800; Leica, Wetzlar, Germany), and sections were thaw-mounted onto Superfrost Plus slides (VWR, Aurora, CO) and then stored at -20°C until needed. Striatal DAT levels were determined by [^{125}I]RTI-55 (PerkinElmer, Waltham, MA) binding, as reported previously (Boja et al. 1992; O'Dell et al. 2011; Pastuzyn et al. 2012; Son et al. 2013). The slides were apposed to film (Biomax MR; Eastman Kodak, Rochester, NY, USA) for 24 h and subsequently developed. Images were digitized and average gray values of the DM and DL striata were determined using ImageJ software (NIH, Bethesda, MD, USA). This densitometric analysis was performed in one hemisphere of striatal sections +1.6 and +0.7 mm from bregma. DAT binding was normalized across slides by subtracting the average gray value of the corpus callosum from the values for DM and DL striatum (background

subtraction) for each section. DAT binding in METH-pretreated rats was then converted to percent of average value per brain region in saline-pretreated rats.

Experimental design

FSCV experiments were performed three to four weeks after METH pretreatment. After recording and stimulating electrodes were optimized and electrically evoked responses had stabilized, pre- and post-drug recordings were collected for analyzing pharmacologically evoked phasic DA events (i.e., transients and surges) and measures of presynaptic DA function (e.g., DA release and uptake) assessed from electrically evoked DA signals. RAC (2 mg/kg i.p.) and NOM (20 mg/kg i.p.) were injected in between the 25-min pre-drug and 120-min post-drug periods for monitoring phasic DA events. Stimulus trains were not applied during this 145-min epoch; rather, one recording of DA levels electrically evoked by the 60-Hz, 1-s train was collected at the beginning of the pre-drug period and another at the end of the post-drug period. A total of 15 animals were used for this study ($n = 6$ for saline pretreatment and $n = 9$ for METH pretreatment) for assessment of presynaptic DA function. In all but one animal, DA was recorded in both the DM and DL striatum. In this METH-pretreated animal, only DA in the DM striatum was recorded. There was also one saline-pretreated animal and one METH-pretreated animal for whom analysis of DAT binding was not available.

Statistical Analysis

Data are expressed as mean $\pm$ SEM or median where applicable. Values for DAT binding, $[DA]_{max}$, $[DA]_p$, and V_{max} in Table 1 and values for $[DA]_{max}$, $[DA]_p$, and k in Table 2 were compared by two-way analysis of variation (ANOVA) with pretreatment and striatal sub-region as factors. Values for $[DA]_{max}$, $[DA]_p$, and k in Figure 2 were analyzed by three-way ANOVA with pretreatment, striatal sub-region, and drug cocktail as factors. Distributions of amplitude, duration, and ITI for phasic events were compared using the Kolmogorov-Smirnov two-sample test. These characteristics of phasic events were not normally distributed and could not be transformed as such; thus, they were assessed using the Friedman non-parametric two-way ANOVA by ranks. All statistical analyses were performed by SAS (SAS Institute Inc., Cary, NC, USA), and the significance level was set at $p < 0.05$.

Drugs and chemicals

($\pm$)-Methamphetamine hydrochloride was provided by the National Institute on Drug Abuse (Rockville, MD, USA). All other reagents were purchased from Sigma Chemical Company (St. Louis, MO, USA).

RESULTS

Representative recordings of electrically evoked DA dynamics

Figure 1 shows representative FSCV recordings demonstrating the effects of the RAC-NOM drug cocktail on electrically evoked DA dynamics collected in the DM and DL striatum of saline- and METH-pretreated animals. Voltammograms, whether plotted individually or sequentially, indicated that DA was the origin of the signal in both striatal sub-regions

evoked by electrical stimulation of the medial forebrain bundle, in both saline and METH pretreated rats, and before and after administration of the RAC-NOM drug cocktail. Overall, the magnitude of evoked DA signals in both striatal sub-regions was decreased by METH pretreatment and increased by the RAC-NOM drug cocktail.

Effects of METH pretreatment on DA innervation and presynaptic DA function

Table 1 shows the effects of METH pretreatment on DAT binding determined by autoradiography, and the maximal concentration of DA ($[DA]_{\max}$), DA release ($[DA]_p$), and DA uptake ($V_{\max}$) determined from electrically evoked FSCV recordings. Pre-drug recordings were analyzed by Eq. 1 to construct Table 1. This analysis, which assumes Michaelis-Menten kinetics for DA uptake, has previously worked well for assessing the relationship between DA depletion and presynaptic DA function after 6-hydroxydopamine lesions (Bergstrom and Garris 2003; Bergstrom et al. 2011) and in methamphetamine-induced neurotoxicity (Howard et al. 2011; Howard et al. 2013a). Two-way ANOVA, with striatal sub-region and pretreatment as factors, showed that METH pretreatment significantly decreased DAT binding ($F_{(1,22)} = 41.4, p < 0.0001$), $[DA]_{\max}$ ($F_{(1,25)} = 11.5; p = 0.002$), $[DA]_p$ ($F_{(1,25)} = 31.5; p < 0.0001$), and $V_{\max}$ ($F_{(1,25)} = 30.0; p < 0.0001$). No significant ($p > 0.05$) effects of striatal sub-region and no significant interactions between sub-region and pretreatment indicated that each DA measure was similarly decreased by METH pretreatment in both the DM and DL striatum. The lack of a significant effect of striatal subregion on any measure of DA function is consistent with previous work in drug-naïve animals (Bergstrom and Garris 2003). The degree of DA depletion (~25 to 30%) is in good agreement with our previous work with METH pretreatment (Chapman et al. 2001; Howard et al. 2011; Howard et al. 2013a; Howard et al. 2013b).

Effects of the RAC-NOM drug cocktail on presynaptic DA function

Figure 2 shows the effects of the RAC-NOM drug cocktail on $[DA]_{\max}$, $[DA]_p$, and k for DA uptake in the DM and DL striatum of saline- and METH-pretreated animals. In contrast to Table 1, which analyzed pre-drug recordings with Eq.1, pre- and post-drug recordings were analyzed by Eq. 2 to construct Figure 2. This analysis, which assumes first-order kinetics for DA uptake, was selected to simplify assessing the effects of the drug cocktail on DA uptake. For example, nomifensine increases K_m (Wu et al. 2001a) while haloperidol decreases $V_{\max}$ (Wu et al. 2002), and this uncompetitive inhibition of DA uptake is difficult to assess with voltammetry. Three-way ANOVA, with striatal sub-region, pretreatment, and drug cocktail as factors, was used for statistical analysis. Consistent with blockade of DA autoreceptors and transporters (Wu et al. 2001a; Wu et al. 2002), the RAC-NOM drug cocktail caused a significant increase in $[DA]_{\max}$ ($F_{(1,50)} = 23.2, p < 0.0001$) and $[DA]_p$ ($F_{(1,50)} = 43.0, p < 0.0001$) and a significant decrease in k ($F_{(1,50)} = 20.8, p < 0.0001$). Consistent with DA denervation, METH pretreatment caused a significant decrease in $[DA]_{\max}$ ($F_{(1,50)} = 14.0, p = 0.0005$) and $[DA]_p$ ($F_{(1,50)} = 11.5, p = 0.0014$). In contrast, there was no significant effect of METH pretreatment on k . Additionally, $[DA]_{\max}$ ($F_{(1,50)} = 4.70, p = 0.035$) and $[DA]_p$ ($F_{(1,50)} = 7.41, p = 0.0089$) were significantly greater in the DM compared to the DL striatum, but k was not significantly different in the two striatal sub-regions. The only significant interaction was for $[DA]_{\max}$ between pretreatment x drug

cocktail ($F_{(2,50)} = 6.49$, $p = 0.014$), suggesting a lesser effect of the drug cocktail in METH-pretreated rats.

To further address specific group effects of the RAC-NOM drug cocktail, drug-induced changes in presynaptic DA function were normalized to percent of the pre-drug response and statistically analyzed using a two-way ANOVA with striatal sub-region and pretreatment as factors. Normalization facilitates group comparisons, because animals act as their own control, and data reflect relative drug effects. This normalization also reduces the number of main effects to two, thus permitting analysis of the effects of the drug cocktail on presynaptic DA function according to striatal sub-region and methamphetamine pretreatment. Such an analysis is not possible in Figure 2, because the three-way ANOVAs found mainly significant main effects (i.e., drug cocktail, striatal sub-region, and METH pretreatment) with only one significant interaction (pretreatment $\times$ drug cocktail for $[DA]_{\max}$) for the entire data set. As shown in Table 2, normalized post-drug values for $[DA]_{\max}$ and $[DA]_p$ were above 100 % (474 to 740 %), which is consistent with the increase in absolute values for these DA measures as shown in Figure 2. Similarly, normalized values for k were below 100 % (40 to 71 %), which is consistent with the decrease in absolute values for this DA measure also as shown in Figure 2. However, there were no significant ($p > 0.05$) effects of striatal sub-region or pretreatment on normalized values for $[DA]_{\max}$, $[DA]_p$ and k and no significant interactions. Overall, the analysis of normalized data suggested that the RAC-NOM drug cocktail elicits similar relative effects on presynaptic DA function in the DM and DL striatum of saline- and METH-pretreated animals.

Representative recordings of phasic DA events

Figure 3 shows representative recordings demonstrating phasic DA events elicited by the RAC-NOM drug cocktail in the DM and DL striatum of saline- and METH-pretreated animals. Two types of phasic responses were observed. The shorter duration transients exhibited a single peak, whereas the longer duration “surges” typically exhibited several, overlapping peaks. Figure 3A and B show recordings in DM and DL striata, respectively, containing only DA transients, whereas Figure 3C and D show recordings in DM and DL striata, respectively, containing both DA transients and surges. Thus, DA transients can occur in isolation or close together in time with surges in the DM and DL striatum of saline- and METH-pretreated animals. Voltammograms, whether plotted individually or sequentially, indicated that DA was the origin of transients and surges in both striatal sub-regions and in both saline and METH pretreatments. Overall, the magnitude of DA transients and surges was decreased by METH pretreatment and was higher in the DM compared to the DL striatum.

Time course of non-electrically evoked phasic DA events

Figure 4 shows the time course of phasic DA events elicited by the RAC-NOM drug cocktail. The combined number of DA transients and surges was plotted in 5-min bins. No phasic DA events were recorded prior to administration of the cocktail. There was a similar, gradual increase in phasic DA events in both the DM and DL striatum of saline- and METH-pretreated animals that reached a plateau approximately 25 to 40 min after drug administration and stayed at this maximal level for the entire 120-min duration of the post-

drug recording period. This result highlights the robust activation of phasic DA signaling by the RAC-NOM drug cocktail. Effects of the drug cocktail in the DM striatum of saline-pretreated animals appear qualitatively similar to that previously observed in drug-naïve animals (Venton and Wightman 2007). Overall, while there was similar activation of phasic DA events in saline- and METH-pretreatment animals, there appeared to be a greater activation in the DM compared to the DL striatum. In the next section, we parse these phasic DA events into transients and surges and statistically analyze individual descriptive characteristics. Transients and surges were divided into separate groups for analysis, because of concern that documented effects of METH pretreatment on DA transients (Howard et al. 2013a) might be obscured in the more complex DA surges.

Characterization of DA transients and surges

Figure 5 shows histograms for amplitude, duration, and ITI of DA transients elicited by the RAC-NOM drug cocktail in the DM and DL striatum of saline- and METH-pretreated animals. The Kolmogorov-Smirnov two-sample test found that METH pretreatment significantly ($p < 0.05$) altered the distribution for each descriptive characteristic in each striatal sub-region. Visualization of the distributions indicated that the most prominent effects of METH pretreatment were decreased amplitude in both DM and DL (i.e., a shift in the distribution to the left) and an increased duration and ITI in DM (i.e., shift in the distribution to the right). Median values for each descriptive characteristic (INSETs) showed a similar phenomenon. To further assess the effects of METH pretreatment and striatal sub-region on DA transients, data for each characteristic were analyzed using a non-parametric two-way ANOVA. This analysis found a significant ($p < 0.05$) effect of striatal sub-region and pretreatment and a significant ($p < 0.05$) interaction for amplitude, duration and ITI (see figure legend for details). *Post hoc* analysis of median values found that all comparisons were significantly ($p < 0.05$) different for each descriptive characteristic of DA transients. The significant effect of METH pretreatment in both striatal sub-regions for each descriptive characteristic is consistent with the analysis of distributions. Overall for DA transients, amplitude was higher, duration was shorter, and ITI was lower (i.e., frequency was higher) in the DM compared to the DL striatum of saline-pretreated animals, and the most robust effect of METH pretreatment across striatal sub-region was decreased amplitude.

Figure 6 shows histograms for amplitude, duration, and ITI of DA surges elicited by the RAC-NOM drug cocktail in the DM and DL striatum of saline- and METH-pretreated animals. The Kolmogorov-Smirnov two-sample test found that METH pretreatment significantly ($p < 0.05$) altered the distribution of amplitude in the DM and DL striatum and duration in the DM striatum. Distributions of duration in the DL striatum and ITI in both the DM and DL striatum were not significantly ($p > 0.05$) altered by METH pretreatment. In general, median values (INSETs) indicated similar effects. The non-parametric two-way ANOVA with sub-region and pretreatment as factors found that the amplitude of DA surges was significantly ($p < 0.05$) higher in the DM compared to the DL striatum and was significantly ($p < 0.05$) decreased by METH pretreatment (see figure legend for details). The interaction between sub-region x pretreatment for amplitude was not significant ($p > 0.05$). The duration of DA surges was significantly ($p < 0.05$) longer in the DM compared to the DL striatum and was significantly ($p < 0.05$) decreased by METH pretreatment. The

interaction between sub-region x pretreatment for duration was significant ($p < 0.05$), and posthoc analysis found that duration was significantly ($p < 0.05$) longer in the DM compared to the DL striatum in saline-pretreated animals and was significantly ($p < 0.05$) decreased by METH pretreatment in the DM striatum. The ITI for DA surges also was significantly ($p < 0.05$) greater in the DM compared to the DL striatum, while there was no significant effect of pretreatment ($p > 0.05$) and no significant interaction ($p > 0.05$). Overall for DA surges, the most prominent difference between striatal sub-regions in saline-pretreated animals was higher amplitude in the DM compared to the DL striatum, and the most prominent effect of METH pretreatment was decreased amplitude and duration in the DM striatum.

DISCUSSION

Pharmacologically evoked DA transients

The present approach, using a drug cocktail consisting of a D2 DA receptor antagonist and DAT inhibitor administered to urethane-anesthetized animals (Venton and Wightman 2007; Park et al. 2010; Park et al. 2011), confers three important advantages to investigating phasic DA signaling: (1) large, high-quality data sets; (2) DA transients elicited by endogenous burst firing; and (3) intrastriatal comparisons in the same animal. The large, high-quality data sets are made possible by the robust pharmacological activation of DA transients in both the DM and DL striatum and the low-noise, movement artifact-free recordings afforded by the anesthetized preparation. The RAC-NOM drug cocktail is necessary to overcome blunting of burst firing of DA neurons by anesthesia (Kelland et al. 1990; Koulchitsky et al. 2012). The pharmacologically evoked activity is also greater than that observed for spontaneously active transients in awake, freely behaving animals and similar to and even exceeding that observed after administration of DAT-inhibiting psychostimulants, nicotine, ethanol, or cannabinoids (Robinson and Wightman 2004; Cheer et al. 2004; Wightman et al. 2007; Cheer et al. 2007; Aragona et al. 2008; Robinson et al. 2009).

The relevance of using the drug cocktail could be questioned on the basis of such a robust activation. However, as we elaborate in the next two sections, the finding that this pharmacological approach demonstrated similar effects on DA transients to those observed in awake, freely behaving animals argues against such a proposition. This result is not entirely unexpected, as both pharmacologically evoked (Shi et al. 2000; Shi et al. 2004) and naturally occurring (Somers et al. 2009; Owesson-White et al. 2012) DA transients are elicited by burst firing of DA neurons. Thus, both types of DA transients may be under similar circuit-level control, which is not the case for electrically evoked phasic-like DA signals, which have a primary reliance on presynaptic mechanisms of DA release and uptake (Bergstrom and Garris 2003).

There is also the issue of whether the observed increase in the frequency of DA transients evoked by the drug cocktail is due to enhanced burst firing by DA neurons or more transients reaching the detection threshold as a result of increased DA release and decreased DA uptake elevating transient amplitude. In the anesthetized preparation, for example, burst firing (Shi et al. 2000) and DA transients (Venton and Wightman 2007) are infrequently observed and minimally, if at all, activated by a D2 DA antagonist and inhibitor of DA uptake when administered separately. Only when the two drugs are combined is there a

robust activation of burst firing and DA transients. Thus, burst firing and DA transients appear to respond similarly to these drugs when administered either individually or combined. A contribution of cocktail-induced elevation of transient amplitude leading to an increase in transient frequency cannot be ruled out. However, if electrically evoked DA signals are indicative of DA transients, then a D2 DA antagonist and an inhibitor of DA uptake should increase transient frequency via increased amplitude when administered individually (Wu et al. 2001a; Wu et al. 2002), which they do not.

Intrastriatal heterogeneity in the regulation of DA transients

Pharmacologically evoked DA transients were larger, more frequent and better temporally resolved (i.e., narrower) in the DM compared to the DL striatum of intact animals. Interestingly, these pronounced differences in transient activity were not accompanied by equivalent differences in either pre- or post-drug values for DA release and uptake. Taken together, these results suggested that observed intrastriatal differences in transient activity are independent of these presynaptic mechanisms. Although most voltammetric studies analyzing electrically evoked DA levels and DA release and uptake in the dorsal striatum have targeted the more medial aspects, the available studies directly comparing the DM and DL striatum have typically failed to document differences in these measurements of presynaptic DA function (Bergstrom and Garris 2003; Howard et al. 2011; Bergstrom et al. 2011). The one exception appears to be Brown et al. (2011), which showed individual recordings reflecting a lower level of electrically evoked DA in the DL compared to the DM striatum, although no averages were reported. Because there is substantial evidence for distinct presynaptic DA function in the DM striatum and nucleus accumbens core and shell and its pharmacological control (e.g., Garris and Wightman 1994; Cragg et al. 2000; Aragona et al. 2008; Zhang et al. 2009a; Zhang et al. 2009b; Avelar et al. 2013), further work needs to establish the precise nature of these mechanisms in the DL striatum.

While measures of presynaptic DA function did not predict regional differences in the activity of pharmacologically evoked DA transients, there may be a precedent for this phenomenon in naturally occurring DA transients recorded in awake, freely behaving animals. For example, spontaneously active DA transients, which are not ostensibly associated with environmental stimuli, are readily observed in the DM and throughout the ventral striatum (Robinson et al. 2002; Stuber et al. 2005b; Wightman et al. 2007; Aragona et al. 2008; Daberkow et al. 2013; Howard et al. 2013a), but have not been reported in the DL striatum to our knowledge. Further study will be required to determine whether these phasic DA signals are indeed less active in this latter striatal sub-region. Moreover, unexpected food reward and a predictive cue elicit DA transients in the DM striatum and nucleus accumbens, but not in the DL striatum (Brown et al. 2011). To date, cue-evoked DA transients have been observed in the DL striatum only after cocaine self-administration is established, whereas they occur in the ventral striatum of the same animals initially and throughout this paradigm (Willuhn et al. 2012). Extended DA signals associated with distant reward also appear to be smaller and delayed in time in the DL striatum compared to the ventromedial striatum (Howe et al. 2013). Taken together, these studies suggest a distinct circuit-level regulation of naturally occurring DA transients in the DL striatum that may be related to the role of this striatal sub-region in habit formation and compulsive drug use

(Everitt and Robbins 2005; Yin and Knowlton 2006). Another possibility is that heterogeneity of DA neurons innervating sub-regions of the striatum (Lammel et al. 2008) may contribute to observed differences in the activity of DA transients in the DM and DL striatum. For example, reduced somatodendritic autoreceptor control of DA neurons innervating the nucleus accumbens shell is proposed to mediate the greater activation of cocaine-induced DA transients here versus the nucleus accumbens core (Aragona et al. 2008). However, to the best of our knowledge, we are not aware of differences in the functional properties of DA neurons innervating the DM versus DL striatum that would account for observed differences in transient activity. It is thus interesting to speculate that our measurements of pharmacologically evoked DA transients in anesthetized rats reflect a functionally relevant reduced baseline state for generating phasic DA signaling in the DL striatum.

METH-induced neurotoxicity disrupts phasic DA signaling

Consistent with findings in our previous studies of electrically evoked phasic-like DA signals and spontaneously active DA transients (Howard et al. 2011; Howard et al. 2013a), the present study demonstrates a disruption of pharmacologically evoked DA transients in rats with METH-induced neurotoxicity. The most prominent effect across striatal sub-regions was decreased transient amplitude. This reduction was proportionally similar in both the DM and DL striatum and comparable to DA depletion indexed by DAT binding. METH pretreatment also altered all transient characteristics in the DM striatum, such that this striatal sub-region more resembled the DL striatum of intact animals, which exhibited a reduced capacity for generating phasic DA signaling. Interestingly, we found a similar disruption of spontaneously active DA transients in the DM striatum of awake, freely behaving rats pretreated with METH (Howard et al. 2013a). As noted above, this result suggests that observed alterations in pharmacologically evoked DA transients in anesthetized rats with METH-induced neurotoxicity reflect the status of naturally occurring phasic DA signaling.

We have previously argued that the reduced amplitude of DA transients in rats with METH-induced DA depletions is due to decreased DA release (Howard et al. 2013a) and is mechanistically similar to the effects of decreased DA release on electrically evoked phasic-like DA signals following DA depletion in METH-induced neurotoxicity and experimental parkinsonism (Garris et al. 1997; Bergstrom and Garris 2003; Howard et al. 2011; Bergstrom et al. 2011). The present study further supports this contention, as the decrease in pharmacologically evoked DA transients in both striatal sub-regions was accompanied by decreased DA release. In contrast, observed decreases in DA uptake should increase the amplitude of DA transients (Robinson and Wightman 2004; Wightman et al. 2007), and thus changes in uptake are not thought to contribute to the observed decreases in transient amplitude. It is also unlikely that changes in the number or frequency of action potentials within a burst contribute to the decrease in transient amplitude, because phasic firing by DA neurons is unaltered until DA depletion is much greater than what is observed here (>96 %; Hollerman and Grace 1990; Harden and Grace 1995).

The diminished amplitude of DA transients in the DM and DL striatum, which we have demonstrated here and elsewhere (Howard et al. 2013a), may have important implications for basal ganglia function and behavior. For example, *preprotachykinin* mRNA expression in the dorsal striatum that is reduced by METH pretreatment is restored by phasic-like electrical stimulation of DA neurons, suggesting a link between disrupted phasic DA signaling and altered functioning of striatal efferent neurons, particularly striatonigral neurons (Howard et al. 2013b). In METH-induced neurotoxicity, moreover, the correlation between *Arc* expression in the DM striatum and reversal learning seen in normal animals is lost (Daberkow et al. 2007; Daberkow et al. 2008), and the degree of impairment of *Arc* regulation in striatal neurons is significantly correlated to the degree of METH-induced DA loss (Barker-Haliski et al. 2012). Interestingly, prior work from our lab (Bergstrom and Garris 2003) has shown that the degree of DA loss correlates with the degree of impairment in phasic DA signaling. In the context of METH-induced neurotoxicity, these impairments in phasic DA signaling are associated with deficits in a number of striatally-mediated learning and memory functions, including sequential motor learning (Chapman et al. 2001; Daberkow et al. 2005), response-reversal learning (Daberkow et al. 2008; Pastuzyn et al. 2012), and formation of stimulus-response associations (Son et al. 2011). These findings suggest that pharmacological approaches to restore phasic DA signaling may be useful in mitigating the behavioral/cognitive sequelae in individuals with a history of METH abuse.

DA “surges”

We also observed phasic events longer than DA transients that we called DA “surges”. The origin of DA surges appears to be multiple DA transients sufficiently overlapping in time to create longer phasic DA signals, because surge-like DA signals appear after NMDA activation of DA cell bodies (Sombers et al. 2009). The present DA surges observed under anesthesia and elicited by the RAC-NOM-drug cocktail may have parallels in behaviorally relevant conditions. Prolonged DA signaling related to reward uncertainty (Fiorillo et al. 2003), distant reward (Howe et al. 2013), and reward seeking (Wassum et al. 2013) may mirror the DA surges described herein. Moreover, spontaneously active surge-like DA signals are recorded in the nucleus accumbens shell of drug-naïve, freely behaving animals (unpublished data). Taken together, these results suggest a physiological role for DA surges. Additionally, DA surges may play an important role in mediating the effects of psychostimulants. Indeed, surge-like DA signals, with durations greater than 10 s, have been reported during cocaine self-administration time-locked to the operant response (Phillips et al. 2003; Stuber et al. 2005a; Stuber et al. 2005b). Also, non-contingent i.v. administration of abused drugs, such as cocaine, nicotine, and ethanol, initially generates an extracellular DA profile consistent with the merging of individual DA transients into a more sustained response (Heien et al. 2005; Cheer et al. 2007). Similar to transients, surge activity was greater in the DM compared to the DL striatum and disrupted by METH pretreatment, although overall to a lesser degree. Presumably, the decrements that we observed for the amplitude of both DA transients and surges intrastrially and in METH-induced neurotoxicity have comparable functional implications.

Conclusions

Measurement of DA transients elicited by administration of the RAC-NOM drug cocktail in urethane-anesthetized rats via FSCV was demonstrated to be a viable approach for assessing phasic DA signaling in METH-induced neurotoxicity. Indeed, the effects of METH pretreatment on DA transients elicited pharmacologically and observed here were similar to those reported previously for spontaneously active DA transients monitored in awake, freely behaving rats. Moreover, the present approach additionally revealed the novel finding of distinct intrastriatal regulation of phasic DA signaling, which was not observed with electrically evoked phasic-like signals and which may parallel naturally occurring DA transients and functional differences between striatal sub-regions. Overall, the present results further support the disruption of phasic DA signaling as a consequential outcome in both METH-induced neurotoxicity (Howard et al. 2011; Howard et al. 2013a) and Parkinson's disease (Garris et al. 1997; Bergstrom and Garris 2003; Schonberg et al. 2010; Bergstrom et al. 2011). This disruption may also provide a putative link between DA depletion and cognitive deficits in these two neuropathological conditions (Nordahl et al. 2003; Frank et al. 2004; Scott et al. 2007; Sandberg and Phillips 2009; Wiecki and Frank 2010; Marshall and O'Dell 2012) as well as others, such as traumatic brain injury (Bales et al. 2009; Bales et al. 2010).

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Figure 1. Representative recordings of electrically evoked DA levels

Panels show FSCV data collected before (Pre-drug) and after (Post-drug) administration of the RAC-NOM cocktail in the DM (**A. Saline DM**) and DL (**B. Saline DL**) striatum of saline-pretreated animals and in the DM (**C. METH DM**) and DL (**B. METH DL**) striatum of METH-pretreated animals. For each evoked recording (solid line), current was monitored at the peak oxidative potential for DA ($\sim +0.6$ V vs Ag/AgCl) and converted to concentration by post-calibration. Application of the 60-Hz stimulus pulse train is demarcated by the short solid line underneath each recording. All background-subtracted cyclic voltammograms collected during the evoked recording are shown sequentially in time by the three-dimensional plot underneath each evoked recording. The x axis is time (s), the y axis is applied potential (V), and z axis is measured current (nA) graphed with a pseudocolor scale. The **INSET** shows an individual background-subtracted cyclic voltammogram calculated as the difference between cyclic voltammograms collected at the maximal amplitude of the evoked response and immediately before stimulation.

Figure 2. Effects of METH pretreatment on measures of presynaptic DA function
 $[DA]_{max}$ for the evoked DA recording, $[DA]_p$ for DA release, and k for DA uptake are shown in **A. Evoked DA**, **B. DA release**, and **C. DA uptake**, respectively. The DM and DL striatum are shown in the top and bottom rows, respectively. Data are expressed as the mean $\pm$ SEM ($n = 6$ to 9). A three-way ANOVA was performed on data in each panel (A., B., and C.), and all significant effects are identified in each panel (see text for details).

Figure 3. Representative recordings of DA transients and surges

Panels show FSCV data collected in saline-pretreated (left) and METH-pretreated (right) animals for DA transients (denoted by asterisks) collected in the DM (A) and DL (B) striatum and DA surges (denoted by bracketed lines) collected in the DM (C) and DL (D) striatum. See Figure 1 for details.

Figure 4. Time course of phasic DA events

A. DM striatum. B. DL striatum. Saline- and METH-pretreated animals are shown in left and right panels, respectively. The number of phasic DA events, consisting of both DA transients and surges, was determined for 5-min bins and averaged across animals. As indicated by the arrow, the RAC-NOM drug cocktail was injected at time 0 min. Data are expressed as mean $\pm$ SEM (n = 6 to 9).

shown by the bracket. A significant ($p < 0.05$) posthoc effect of METH pretreatment in each striatal sub-region is shown by an asterisk.

Figure 6. Histograms of characteristics for DA surges

A. Amplitude. B. Duration. C. ITI. Each histogram shows an individual characteristic for all DA surges collected in the DM (left panels) and DL (right panels) striatum of saline- and METH-pretreated animals. Bin size is 15 nM for amplitude, 0.5 sec for duration, and 10 sec for ITI. Counts (N) in each bin are normalized to the total number of counts (N_{total}). The total number of DA surges analyzed in the DM striatum of saline- and METH pretreated animals was 651 and 957, respectively, and in the DL striatum of saline- and METH pretreated animals was 968 and 1,486, respectively. **INSET.** Median values are shown for surge characteristics determined in the DM and DL striatum of saline- and METH-pretreated animals. According to the Friedman non-parametric two-way ANOVA by ranks, amplitude was significantly decreased by METH pretreatment ($F_{(1,4058)} = 40.0, p < 0.0001$) and significantly greater in the DM compared to the DL striatum ($F_{(1,4058)} = 452.4, p < 0.0001$). There was no significant interaction between sub-region x pretreatment. Significant effects of pretreatment ($F_{(1,4058)} = 37.5, p < 0.0001$), striatal sub-region ($F_{(1,4058)} = 391.0, p < 0.0001$) and a significant interaction between sub-region x pretreatment ($F_{(2,4058)} = 33.5, p < 0.0001$) were found for duration. There was a significant effect of striatal sub-region for ITI ($F_{(2,4029)} = 441.1; p < 0.0001$); however, no significant effects of pretreatment or interaction were found. Portions of the statistical analysis are graphically represented (see text for details). A significant ($p < 0.05$) main effect of striatal sub-region is shown by the bracket. A

significant ($p < 0.05$) posthoc effect of METH pretreatment in each striatal sub-region is shown by an asterisk.

Table 1

Effects of METH pretreatment on measures of DA depletion and presynaptic DA function.

	DA measures							
	DAT Binding (%)		[DA] _{max} (μM)		[DA] _p (μM)		V _{max} (μM/s)	
	Saline	METH	Saline	METH	Saline	METH	Saline	METH
Striatal sub-region								
DM	100.00±2.89	71.40±4.17	0.72±0.26	0.21±0.05	0.027±0.005	0.007±0.001	1.85±0.38	0.52±0.07
DL	100.00±4.34	74.26±4.22	0.54±0.17	0.11±0.01	0.018±0.005	0.005±0.001	1.28±0.27	0.43±0.03

Data are expressed as mean±SEM.

Table 2

Effects of the RAC-NOM drug cocktail on measures of presynaptic DA function.

	DA measures					
	[DA] _{max}		[DA] _p		k	
	Saline	METH	Saline	METH	Saline	METH
Striatal sub-region						
DM	728±123	740±113	558±111	753±203	50±11	71±21
DL	575±159	710±62	474±129	761±120	40±7	62±7

Data are expressed as a percent of pre-drug and as mean±SEM.