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AMPHETAMINE AUGMENTS VESICULAR DOPAMINE RELEASE IN THE DORSAL AND VENTRAL STRIATUM THROUGH DIFFERENT MECHANISMS

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Abstract

Amphetamine has well-established actions on presynaptic dopamine signaling, such as inhibiting uptake and degradation, activating synthesis, depleting vesicular stores, and promoting dopamine-transporter reversal and non-exocytotic release. Recent *in vivo* studies have identified an additional mechanism: augmenting vesicular release. Here we investigated how amphetamine elicits this effect. Our hypothesis was that amphetamine enhances vesicular dopamine release in dorsal and ventral striata by differentially targeting dopamine synthesis and degradation. In urethane-anesthetized rats, we employed voltammetry to monitor dopamine, electrical stimulation to deplete stores or assess vesicular release and uptake, and pharmacology to isolate degradation and synthesis. While amphetamine increased electrically evoked dopamine levels, inhibited uptake, and up-regulated vesicular release in both striatal sub-regions in controls, this psychostimulant elicited region-specific effects on evoked levels and vesicular release but not uptake in drug treatments. Evoked levels better correlated with vesicular release compared to uptake, supporting enhanced vesicular release as an important amphetamine mechanism. Taken together, these results suggested that amphetamine enhances vesicular release in the dorsal striatum by activating dopamine synthesis and inhibiting dopamine degradation, but targeting an alternative mechanism in the ventral striatum. Region-distinct activation of vesicular dopamine release highlights complex cellular actions of amphetamine and may have implications for its behavioral effects.

Keywords

exocytosis; degradation; synthesis; voltammetry; rat

INTRODUCTION

Substantive evidence indicates that amphetamine (AMPH), a highly addictive drug of abuse exhibiting clinical efficacy for treating narcolepsy and attention deficit hyperactivity

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disorder (Heal *et al.* 2009; Peacock and Benca 2010), targets presynaptic dopamine (DA) signaling. Effects include inhibiting the dopamine transporter (DAT) and monoamine oxidase and activating tyrosine hydroxylase, but depleting vesicular DA stores and promoting non-exocytotic DA release via DAT reversal are considered primary (Fleckenstein *et al.* 2007; Sulzer 2011). More recently, AMPH has been shown to augment vesicular DA release in both dorsal and ventral striata *in vivo* (Ramsson *et al.* 2011b; Daberkow *et al.* 2013). While the significance of this unexpected finding to overall drug effect remains to be determined, enhanced vesicular DA release may drive AMPH-induced increases in phasic DA signaling (Ramsson *et al.* 2011b; Daberkow *et al.* 2013), which is important for reinforcement-learning in goal-directed behavior and addiction (Hyman 2005; Wanat *et al.* 2009). Several other DAT inhibitors have also been shown to increase vesicular DA release (Ewing *et al.* 1983; Kuhr *et al.* 1986; Jones *et al.* 1995; Lee *et al.* 2001; Venton *et al.* 2006; Oleson *et al.* 2009; Kile *et al.* 2010; Chadchankar *et al.* 2012), suggesting a common action for a major psychostimulant class.

How AMPH augments vesicular DA release is unknown, but potential mechanisms are suggested by other DAT inhibitors. Cocaine and methylphenidate act on DA storage pools associated with synapsin (Venton *et al.* 2006; Kile *et al.* 2010) and α -synuclein (Chadchankar *et al.* 2012), respectively. Several DAT inhibitors re-distribute cytosolic and membrane-bound vesicles (Riddle *et al.* 2002; Riddle *et al.* 2007; Volz *et al.* 2007) and increase vesicular DA uptake (Brown *et al.* 2001; Volz *et al.* 2008). As a drug with complex actions, AMPH could exert additional, unique effects, including the inhibition of DA degradation (Scorza *et al.* 1997) and activation of DA synthesis (Kuczenski 1975) leading to elevated cytosolic DA levels and vesicular packaging, promoting exocytosis by liberating intracellular Ca^{2+} stores (Mundorf *et al.* 1999), and increasing membrane excitability as a DAT substrate (Ingram *et al.* 2002).

The present study used voltammetry and electrical stimulation to investigate the mechanism by which AMPH augments vesicular DA release in dorsal and ventral striata *in vivo*. We employed an experimental design previously used to demonstrate that amfonelic acid and cocaine reinstate vesicular DA release after its near-complete depletion (Ewing *et al.* 1983; Kuhr *et al.* 1986; Venton *et al.* 2006). These results were interpreted as the two psychostimulants mobilizing the reserve DA pool to replenish the readily releasable DA pool independently of an action on DA synthesis, because tyrosine hydroxylase was pharmacologically blocked. However, vesicular mobilization was not directly assessed and thus not proven. We selected this design, because the robust response serves as a gauge of AMPH's effectiveness and because amfonelic acid and cocaine are perhaps the best-established DAT inhibitors for up-regulating vesicular DA release. Indeed, amfonelic acid has been recognized for decades as an archetypal enhancer of vesicular release (Aceto *et al.* 1970; Shore 1976), and this cocaine effect manifests across brain-slice (Jones *et al.* 1995; Lee *et al.* 2001; Kile *et al.* 2010), anesthetized (Ramsson *et al.* 2011b), and awake (Oleson *et al.* 2009) preparations. Because AMPH could conceivably act by inhibiting DA degradation, in addition to activating DA synthesis, we modified the design to also incorporate pharmacological blockade of monoamine oxidase, in order to assess the respective contributions of both presynaptic mechanisms. The experimental design also permitted resolving the respective contributions of vesicular DA release and DA uptake to observed AMPH-induced changes in electrically evoked DA levels. The hypothesis tested was that AMPH distinctly up-regulates vesicular DA release in striatal sub-regions by differentially targeting DA synthesis and degradation. Our results are consistent with a mechanism of AMPH action characterized by generalized uptake inhibition and up-regulation of vesicular release across striatal sub-regions, but a specific degradation- and synthesis-sensitive enhancement of vesicular release in the dorsal striatum only.

MATERIALS AND METHODS

Animals

Adult, male Sprague-Dawley rats (281 to 443 g) were purchased from Harlan Industries (Indianapolis, IN, USA). Animals were housed in a temperature-controlled vivarium, with a 12 hr light/dark cycle and *ad libitum* food and water. All procedures in this study follow the *NIH Guide for the Care and Use of Laboratory Animals* and were approved by the Illinois State University Institutional Animal Care and Use Committee.

Drugs

Urethane, alpha-methyl-DL-tyrosine-methyl ester hydrochloride (α MpT), pargyline hydrochloride, and D-amphetamine sulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in 0.9% sterile saline.

Surgery

Rats were anesthetized with urethane (1.5 g/kg i.p.) and immobilized in a stereotaxic frame (Kopf Instrumentation, Tujunga, CA, USA) (Ramsson *et al.* 2011a; Ramsson *et al.* 2011b). Holes were drilled in the skull to position recording, stimulating and reference electrodes. Recording electrodes targeted the dorsal (AP +1.2, ML -2.0, DV -4.5; 0 ° angle) and ventral (AP +1.4, ML +3.0, DV -6.6; 12 ° angle) striatum. The stimulating electrode was directed towards the medial forebrain bundle (AP -4.6, ML +1.4, DV -7.0; 0 ° angle) ipsilateral to recording electrodes. The silver/silver chloride reference electrode was positioned in contralateral superficial cortex. All coordinates are from a flat-skull brain atlas (Paxinos and Watson 1986). After the initial placement, the stimulating and recording electrodes were incrementally lowered to locate sites supporting robust levels of electrically evoked DA. After this optimization, electrode positions were not changed for the duration of the experiment.

Fast-scan Cyclic Voltammetry

Fast-scan cyclic voltammetry recorded DA at a carbon-fiber microelectrode (Ramsson *et al.* 2011a; Ramsson *et al.* 2011b). This recording electrode was fabricated by aspirating a single carbon fiber (3.5 μ m radius; Cytec Engineering Materials, West Patterson, NJ, USA) into a borosilicate capillary tube (1.2 mm o.d.; Sutter Instrument, Novato, CA, USA) and pulling to a taper using a micropipette puller (Narishige, Tokyo, Japan). The end of the carbon fiber protruding beyond the glass insulation was cut to a length of ~100 μ m. Fast-scan cyclic voltammetry (-0.4 to 1.3 V, 400 V/s, 10 Hz) was performed by a UEI bipotentiostat (Department of Chemistry Electronics Facility, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA) and controlled by TH-1 software (ESA, Chelmsford, MA, USA). Current recorded at the peak oxidative potential for DA (~0.6 V) was converted to DA concentration using a post-calibration factor obtained from flow injection analysis (Logman *et al.* 2000). DA was identified by the background-subtracted cyclic voltammogram (Michael *et al.* 1998).

Electrical Stimulation

Electrical stimulation was computer generated and consisted of optically isolated, constant-current (Neurolog NL800, Digitimer, Letchworth Garden City, UK) biphasic pulses (300 μ A and 2 ms each phase). Tips of the twisted bipolar stimulating electrode (Plastics One, Roanoke, VA, USA) were separated by ~1 mm. Two types of stimulus trains were applied (Venton *et al.* 2006). The supraphysiological depleting stimulation (60 Hz, 10 s) was used to disrupt the readily releasable pool for DA. A more-physiological stimulation (60 Hz, 0.4 s) was used to assess the effects of AMPH on vesicular DA release. This stimulation is referred

to here as reinforcing-like, because frequency, duration, and pulse width are identical to trains that animals will lever press to obtain during the operant paradigm of intracranial self-stimulation (Cheer *et al.* 2007). However, a higher current intensity (± 300 versus $125 \mu\text{A}$) was used in the present study, because anesthesia blunts the amplitude of electrically evoked DA levels (Garris *et al.* 1997). This reinforcing-like stimulation is also highly efficacious in revealing the augmentation of vesicular DA release by cocaine and AMPH *in vivo* (Venton *et al.* 2006; Oleson *et al.* 2009; Ramsson *et al.* 2011b; Daberkow *et al.* 2013).

Experimental Design

The experimental design, modified from Venton *et al.* (2006), is shown in Figure 1. There were 3 periods, pre-treatment, treatment, and post-treatment. The pre-treatment period (–10 to 0 min) established baseline measurements of DA to evaluate treatment effects. A total of 5 reinforcing-like trains were applied every 2 min during this period. The treatment period (0 to 100 min) used the depleting stimulation to disrupt vesicular DA release and drugs to block DA synthesis (αMPT ; 200 mg/kg i.p.) and degradation (pargyline; 75 mg/kg i.p.). A total of 5 trains were applied every 20 min during this period. Drugs were injected immediately following the first stimulation. The post-treatment period (100 to 160 min) established post-treatment baseline measurements and examined the effects of AMPH (10 mg/kg i.p.; calculated as free base). A total of 30 reinforcing trains were applied every 2 min during this period. AMPH was administered at 118 min in this period. A total 52 rats were used in this study. There were five treatment groups: (1) no-depleting stimulation control (n = 8); (2) depleting stimulation-only control (n = 12); (3) depleting stimulation with αMPT (n = 7); (4) depleting stimulation with pargyline (n = 13); and (5) depleting stimulation with αMPT and pargyline (n = 12). Although not always successful, simultaneous DA recording was attempted in both the dorsal and ventral striatum of all rats. The AMPH dose was selected, because 10 mg/kg increases vesicular DA release in both the dorsal and ventral striatum of anesthetized animals (Ramsson *et al.* 2011b). While both 1 and 10 mg/kg also elevate vesicular DA release in both striatal sub-regions of awake animals, the low dose is only effective in the ventral striatum under anesthesia (unpublished observations; Daberkow *et al.*, 2013). Additionally, the intense depleting stimulation is contraindicated in awake animals.

Data Analysis

DA signals evoked by the reinforcing-like stimulation were analyzed to determine maximal amplitude ($[\text{DA}]_{\text{max}}$), vesicular DA release, and DA uptake. Analysis of vesicular release and uptake permits resolving the respective contributions of these mechanisms to observed AMPH-induced changes in $[\text{DA}]_{\text{max}}$. Vesicular DA release was calculated as the concentration of DA elicited per stimulus pulse ($[\text{DA}]_{\text{p}}$), and DA uptake was calculated as a first-order rate constant (k) according to:

$$d[\text{DA}]/dt = [\text{DA}]_{\text{p}} * f - k[\text{DA}] \quad \text{Eq. 1}$$

where f is stimulation frequency (Wightman *et al.* 1988). Absolute values for $[\text{DA}]_{\text{max}}$, $[\text{DA}]_{\text{p}}$, and k are reported in Results for the combined pre-treatment data set. To minimize heterogeneity of evoked DA responses recorded in the dorsal and ventral striatum (May and Wightman 1989; Garris *et al.* 1994), data for comparing treatment effects were normalized to a percent of pre-treatment (% pre-treatment) in each animal. We have previously used first-order kinetics to analyze DA uptake in AMPH responses evoked by the same reinforcing-like stimulation (Ramsson *et al.* 2011b; Daberkow *et al.* 2013). The use of first-order kinetics has been previously justified on the basis that the effects of AMPH are uncompetitive (i.e., both K_{m} and V_{max} change) (Fleckenstein *et al.* 1999; Ramsson *et al.* 2011b), which is difficult to assess using Michaelis-Menten kinetics and *in vivo*

voltammetry (Wu *et al.* 2001b), and that evoked DA levels are near or below K_m for DA uptake, which establishes pseudo-first-order conditions (Rammson *et al.* 2011b). This approach has also been shown, under conditions in which uptake inhibition by AMPH should be competitive (i.e., change in K_m), to provide similar determinations of vesicular DA release and simulated curves that well describe data compared to Michaelis-Menten kinetics (Daberkow *et al.* 2013). A nonlinear regression with a simplex minimization algorithm was used to determine best-fit parameters for vesicular DA release and DA uptake (Wu *et al.* 2001b). Responses calculated from Eq. 1 were temporally distorted equivalently before comparing to measured responses.

Statistical Analysis

Repeated measures multivariate analysis of variance (RMANOVA) in SAS (SAS Institute Inc., Cary, NC, USA) was used to assess significant differences in $[DA]_{max}$, $[DA]_p$, and k over time within each treatment group (Figs. 2 to 6). One-way ANOVA with contrasts in SAS was used to assess significant differences in $[DA]_{max}$ across treatment groups during the post-treatment period (Fig. 7). Post hoc testing used a sequential Bonferroni correction for contrasts. Correlations (Fig. 8) were performed by Sigma Plot 12.0 (Systat Software Inc., San Jose, CA, USA). Two-way ANOVA with contrasts in SAS was used to assess significant differences in pre-AMPH baseline $[DA]_{max}$ (Fig. 9). Post hoc testing used Dunnett's test with the no-depleting stimulation treatment serving as the control. Striatal sub-regional differences in the absolute values for $[DA]_{max}$, $[DA]_p$, and k for the pre-treatment condition and described in the text of Results were analyzed in Excel (Microsoft, Redmond, WA) using a two-tailed t-test. The significance level for all comparisons was set at $p = 0.05$.

RESULTS

Controls

Although we have previously demonstrated that AMPH inhibits DA uptake and augments vesicular DA release in both dorsal and ventral striata (Ramsson *et al.* 2011b; Daberkow *et al.* 2013), the present study employed a longer and more complex experimental design with treatments incorporating a depleting stimulation and pharmacological manipulation of DA synthesis and degradation. We thus first needed to establish appropriate controls examining the effects of the supraphysiological depleting stimulation on the AMPH response in the absence of drugs. These controls are shown in Figures 2 and 3.

Figure 2 shows the depleting stimulation-only control. Individual recordings evoked by the reinforcing-like stimulation reflect a decrement after the depleting stimulation and an augmentation after AMPH in both striatal sub-regions (Fig. 2A). Averaged results (Fig. 2B) show similar phenomena. The depleting stimulation alone caused a ~25 % decrease in maximal amplitude ($[DA]_{max}$) in both the dorsal and ventral striatum, although the time courses appeared to differ. AMPH-induced increases in $[DA]_{max}$ evoked by the reinforcing-like stimulation were comparable in both striatal sub-regions. Across all animals in the present study, $[DA]_{max}$ of the pre-treatment response was 261 ± 37 nM ($n = 36$) in the dorsal striatum and 253 ± 36 nM ($n = 28$) in the ventral striatum. These concentrations, which are not significantly different ($p = 0.88$; t-test), fall within the range of naturally occurring phasic DA signals recorded in awake, freely behaving animals (Robinson and Wightman 2007). This result further supports our contention that the reinforcing-like stimulation is physiological.

Data for statistical analysis are displayed in Figure 2C for $[DA]_{max}$ (left), vesicular DA release ($[DA]_p$, middle), and DA uptake (k , right), for three time points of the post-treatment

period. These time points were selected to capture the initial AMPH effect. Parameters for vesicular DA release and DA uptake were calculated from the same responses evoked by the reinforcing-like stimulation from which $[DA]_{\max}$ was determined. For the pre-treatment responses across all animals, k was significantly ($p < 0.0001$; t-test) higher in the dorsal compared to the ventral striatum (1.15 ± 0.08 and $0.69 \pm 0.07 \text{ s}^{-1}$, respectively) but $[DA]_p$ was not different significantly ($p = 0.77$; t-test) between the striatal sub-regions (14.4 ± 2.2 and $13.5 \pm 2.5 \text{ nM}$ respectively). For the depleting stimulation-only control, AMPH increased $[DA]_{\max}$ and vesicular DA release but decreased DA uptake in both striatal sub-regions. Statistical analysis revealed a significant effect of time for $[DA]_{\max}$ ($F_{(2,14)} = 10.54$, $p = 0.0016$), vesicular DA release ($F_{(2,14)} = 7.03$, $p = 0.0077$), and DA uptake ($F_{(2,14)} = 10.80$, $p = 0.0015$). There was a significant effect of region on DA uptake ($F_{(1,4)} = 13.96$, $p = 0.020$) but not for $[DA]_{\max}$ or vesicular DA release. All region*time interactions were not significant. The sample size was $n = 7$ in the dorsal striatum and $n = 6$ in the ventral striatum.

Figure 3 shows the no-depleting stimulation control. Individual (Fig. 3A) and averaged (Fig. 3B) responses demonstrate signal maintenance across the 100 min interval when the various treatments of the depleting stimulation and drugs would be applied and an increase in $[DA]_{\max}$ evoked by the reinforcing-like stimulation following AMPH administration. This increase was more rapid compared to the depleting stimulation-only control (Fig. 2). In both the dorsal and ventral striatum for the no-depleting stimulation control, $[DA]_{\max}$ and vesicular DA release increased and DA uptake decreased after AMPH administration (Fig. 3C). Statistical analysis revealed a significant effect of time for $[DA]_{\max}$ ($F_{(2,8)} = 7.85$, $p = 0.013$), vesicular DA release ($F_{(2,8)} = 5.43$, $p = 0.032$), and DA uptake ($F_{(2,8)} = 64.77$, $p < 0.0001$). There was a significant effect of region on DA uptake ($F_{(1,3)} = 12.97$, $p = 0.037$) but not for $[DA]_{\max}$ or vesicular DA release. All region*time interactions were not significant. The sample size was $n = 5$ in the dorsal striatum and $n = 4$ in the ventral striatum. Overall, the control experiments demonstrated that AMPH elevated electrically evoked DA levels, inhibited DA uptake, and increased vesicular DA release in both striatal sub-regions whether the depleting stimulation was applied or not and that the depleting stimulation delayed the AMPH response.

Blockade of DA Synthesis

Figure 4 shows the effects of the depleting stimulation and αMpT (200 mg/kg i.p.), a tyrosine hydroxylase blocker. This experiment thus examines whether AMPH activation of DA synthesis underlies the AMPH-induced increase in vesicular DA release observed in the depleting stimulation-only control (Fig. 2). This experiment is also identical to the original design of Venton et al. (2006) demonstrating the ability of cocaine to reinstate vesicular DA release after near-complete depletion of the readily releasable DA pool. Individual (Fig. 4A) and averaged (Fig. 4B) responses show that the depleting stimulation and αMpT caused a marked decrease in $[DA]_{\max}$ during the treatment period. AMPH elicited a robust increase in $[DA]_{\max}$ evoked by the reinforcing-like stimulation in the ventral striatum after this treatment. By comparison, the increase was considerable blunted in the dorsal striatum. Data for statistical analysis (Fig. 4C) demonstrated that AMPH caused a greater increase in $[DA]_{\max}$ and vesicular DA release in the ventral versus the dorsal striatum but a similar decrease in DA uptake in both sub-regions. Statistical analysis revealed a significant effect of region and time for both $[DA]_{\max}$ ($F_{(1,5)} = 11.86$, $p = 0.018$ and $F_{(2,10)} = 5.86$, $p = 0.021$, respectively) and vesicular DA release ($F_{(1,5)} = 6.62$, $p = 0.050$, and $F_{(2,10)} = 5.73$, $p = 0.022$, respectively) but only a significant effect of time for DA uptake ($F_{(2,10)} = 10.80$, $p = 0.0032$). All region*time interactions were not significant. The sample size was $n = 6$ in the dorsal striatum and $n = 6$ in the ventral striatum. Overall, this experiment suggests that, while AMPH similarly inhibited DA uptake in both striatal sub-regions after the αMpT

treatment, the AMPH-induced augmentation of electrically evoked DA levels and vesicular DA release is more dependent on DA synthesis in the dorsal compared to the ventral striatum.

Blockade of DA Degradation

Figure 5 shows the effects of the depleting stimulation and pargyline (75 mg/kg i.p.), a monoamine oxidase blocker. This experiment thus examines whether AMPH inhibition of DA degradation underlies the AMPH-induced increase in vesicular DA release observed in the depleting stimulation-only control (Fig. 2). Individual (Fig. 5A) and averaged (Fig. 5B) responses show that the depleting stimulation and pargyline caused a small decrease in $[DA]_{\max}$ during the treatment period. AMPH increased $[DA]_{\max}$ evoked by the reinforcing-like stimulation in the ventral striatum only under these conditions. Data for statistical analysis (Fig. 5C) demonstrate that AMPH increased $[DA]_{\max}$ and vesicular DA release in the ventral but not the dorsal striatum, and decreased DA uptake to a similar degree in both striatal sub-regions. Statistical analysis revealed a significant effect of region for both $[DA]_{\max}$ ($F_{(1,4)} = 45.73$, $p = 0.0025$) and vesicular DA release ($F_{(1,4)} = 45.89$, $p = 0.0025$) but of time for DA uptake ($F_{(2,14)} = 9.33$, $p = 0.0027$). All region*time interactions were not significant. The sample size was $n = 7$ in the dorsal striatum and $n = 6$ in the ventral striatum. Overall, this experiment suggests that, while AMPH similarly inhibited DA uptake in both striatal sub-regions after the pargyline treatment, the AMPH-induced augmentation of electrically evoked DA levels and vesicular DA release is more dependent on DA degradation in the dorsal compared to the ventral striatum.

Combined Blockade of DA Synthesis and Degradation

Figure 6 shows the effects of the depleting stimulation and blockade of DA synthesis with α MpT (200 mg/kg, i.p.) and DA degradation with pargyline (75 mg/kg, i.p.). This experiment thus further examines whether AMPH activation of DA synthesis and inhibition of DA degradation underlie the AMPH-induced increase in vesicular DA release observed in the depleting stimulation-only control (Fig. 2). Individual (Fig. 6A) and averaged (Fig. 6B) responses show that the depleting stimulation with α MpT and pargyline caused a modest decrease in $[DA]_{\max}$ during the treatment period. AMPH increased $[DA]_{\max}$ evoked by the reinforcing-like stimulation in the ventral striatum only under these conditions. Data for statistical analysis (Fig. 6C) demonstrate that AMPH increased $[DA]_{\max}$ and vesicular DA release in the ventral but not the dorsal striatum, and decreased DA uptake to a similar degree in both regions. Statistical analysis revealed a significant effect of region ($F_{(1,5)} = 64.79$, $p = 0.0005$), time ($F_{(2,16)} = 5.45$, $p = 0.0157$), and region*time ($F_{(2,10)} = 6.50$, $p = 0.0155$) on $[DA]_{\max}$, a significant effect of region ($F_{(1,5)} = 49.24$, $p = 0.0009$) and region*time ($F_{(2,10)} = 4.23$, $p = 0.0465$) on vesicular DA release, and a significant effect of time ($F_{(2,16)} = 6.39$, $p = 0.0091$) on DA uptake. The sample size was $n = 10$ in the dorsal striatum and $n = 6$ in the ventral striatum. Overall, this experiment suggests that, while AMPH similarly inhibited DA uptake in both striatal sub-regions after the combined α MpT and pargyline treatment, the AMPH-induced augmentation of electrically evoked DA levels and vesicular DA release is more dependent on DA synthesis and degradation in the dorsal compared to the ventral striatum.

Across-treatment Analysis of AMPH Effects on $[DA]_{\max}$

The previous experiments examined individual treatment effects of AMPH between regions. The goal of this analysis was to compare these effects across treatments. Figure 7 shows $[DA]_{\max}$ evoked by the reinforcing-like stimulation during the post-treatment period. Data are from Figures 2 through 6 and were normalized to the baseline value at time 0 min, which is equivalent to time 118 min of the previous figures. This manipulation was performed to minimize treatment effects on $[DA]_{\max}$. Data collected in the dorsal striatum segregated into

two groups, with AMPH eliciting a robust increase in $[DA]_{\max}$ in controls but having minimal to no effect in the pharmacological treatments (Fig. 7A). Statistical analysis of the last time point (40 min) revealed a significant effect of treatment ($F_{(4,30)} = 6.34$, $p = 0.0008$) and a significant difference between the pooled controls and each of the pharmacological treatments ($p < 0.004$). In the ventral striatum by contrast, data appeared to segregate into a single group, and AMPH elicited a robust increase across treatments (Fig. 7B). Although individual treatments with α MpT and pargyline did not achieve the same AMPH-induced increase in $[DA]_{\max}$ as the two controls or combined drug cocktail, statistical analysis revealed no significant ($F_{(4,23)} = 1.42$, $p = 0.26$) effect of treatment. Figure 7 also showed that the depleting stimulation delayed the AMPH response but did not alter its magnitude in both striatal sub-regions. Overall, this across-treatment analysis suggests that AMPH-induced increases in electrically evoked DA levels are more dependent on DA synthesis and degradation in the dorsal compared to the ventral striatum.

Relationships between $[DA]_{\max}$ and Vesicular DA Release or DA Uptake

Figure 8 shows correlations between vesicular DA release or DA uptake and $[DA]_{\max}$. This analysis was performed, because Figures 2 to 6 suggested that AMPH reduced DA uptake consistently across treatments but elicited variable increases in vesicular DA release that mirrored $[DA]_{\max}$. If $[DA]_{\max}$ better correlates with vesicular DA release than DA uptake, then the pronounced striatal sub-regional differences with $[DA]_{\max}$ shown in Figure 7 also relate more to differences in vesicular DA release than DA uptake. Determining $[DA]_p$ and for all of the responses in Figure 7 would be very time consuming and is thus prohibitive. This correlation analysis could additionally reveal insight into the relative importance of the presynaptic mechanisms of vesicular DA release and DA uptake in mediating AMPH-induced alterations in extracellular DA levels. Data in Figure 8 are the averaged values displayed in Panel C of Figures 2 to 6 and combined across treatment and region. Vesicular DA release was tightly associated with the trend line and significantly correlated ($r = 0.990$; $p < 0.01$) with $[DA]_{\max}$ (Fig. 8A). In contrast, although a significant correlation was found for DA uptake and $[DA]_{\max}$ ($r = 0.484$; $p < 0.05$), data were more diffusely scattered around the trend line (Fig. 8B). Overall, these results suggest that augmented vesicular DA release plays a greater role in increasing electrically evoked DA levels than DA uptake inhibition after AMPH administration.

Treatment Effects on pre-AMPH Baseline $[DA]_{\max}$

Figure 9 compares treatment effects on the pre-AMPH baseline responses to the reinforcing-like stimulation. This analysis was performed, because the analysis of individual treatments in Figures 2 to 6 suggested that treatments elicited variable effects on $[DA]_{\max}$ prior to AMPH administration, which may have altered the AMPH responses shown in Figure 7. Overall, pre-AMPH baseline $[DA]_{\max}$ was altered in a region- and treatment-specific manner. Statistical analysis revealed a significant effect of region ($F_{(1,53)} = 8.80$, $p = 0.0045$) and treatment ($F_{(4,53)} = 18.69$, $p < 0.0001$) but no significant interaction ($F_{(4,53)} = 1.49$, $p = 0.22$). Dunnett's test of the main treatment effects using the no-depleting stimulation group (– stim) as the control revealed a significant decrease by α MpT (α MT; $p < 0.0001$) and increase by pargyline (P; $p = 0.026$). The increase in $[DA]_{\max}$ with pargyline could be due to increased cytosolic DA levels after MAO inhibition leading to greater vesicular packaging and enhanced DA release, consistent with previous studies (Kuhr *et al.* 1986; Garriss and Wightman 1995; Heien *et al.* 2003). There was also a trend for a decrease with depleting stimulation (+ stim; $p = 0.098$) but not for the combined treatment of α MpT and pargyline (α MT, P; $p = 0.91$). The lower $[DA]_{\max}$ in the depleting stimulating-only compared to no-depleting stimulation control may thus be responsible for the delayed AMPH response in the former treatment (Figs. 2 and 3). Treatments with pargyline (P and α MT, P) also appeared to elicit the most prominent differential effects between striatal sub-regions, with greater

increases in the ventral compared to the dorsal striatum (P: 152.8 ± 17.0 and 97.7 ± 13.6 % pre-treatment, respectively; α MT, P: 98.1 ± 14.4 and 62.1 ± 8.9 % pre-treatment, respectively), but these comparisons were not statistically analyzed due to the non-significant interaction. Overall, these results suggest that treatments elicited variable effects on $[DA]_{\max}$, a lower $[DA]_{\max}$ may be responsible for the delayed AMPH response in the depleting-stimulation-only control, and pargyline may be more effective in the ventral compared to the dorsal striatum.

DISCUSSION

The present study investigated how AMPH augments vesicular DA release, a recently identified target of this psychostimulant on presynaptic DA signaling. We showed that AMPH effects on extracellular DA levels evoked by reinforcing-like stimulation are altered by drugs manipulating DA synthesis and degradation in a striatal sub-region-specific manner and that these effects were mediated predominately by changes in vesicular DA release and not DA uptake. Our results are consistent with AMPH enhancing vesicular DA release by activating DA synthesis and inhibiting DA degradation in the dorsal striatum but by targeting an alternative mechanism in the ventral striatum. Overall, this study advances our understanding of the complex cellular actions of AMPH and the hypothesis that effects of abused drugs are regionally distinct in the striatum.

Experimental Design

Two controls, with and without the depleting stimulation, were incorporated into the experimental design. Both controls showed that AMPH increased evoked DA levels, inhibited DA uptake, and up-regulated vesicular DA release in the dorsal and ventral striatum, similar to our previous work (Ramsson *et al.* 2011a,b; Daberkow *et al.* 2013). The depleting stimulation delayed the response of $[DA]_{\max}$ to AMPH but not its magnitude (Fig. 7). This delay could be due to compromised releasable DA stores, because pre-AMPH baseline $[DA]_{\max}$ had not completely rebounded (Figs. 2, 3, and 9). Given a wait time of 5 s per stimulus pulse (Wightman *et al.* 1988), 50 min is required for the releasable pool to recover fully after the 10-s depleting stimulation, and AMPH was injected 40 min after the last train of the treatment period. Also, reinforcing-like trains were applied during the post-treatment period, which would have additionally taxed the releasable pool.

The experimental design also incorporated kinetic analysis that resolved the respective contributions of vesicular DA release and DA uptake to observed AMPH-induced changes in $[DA]_{\max}$. This capability permitted us to identify vesicular release as the predominant contributor to observed treatment effects on AMPH responses. The basis for this conclusion is two-fold. First, AMPH effects on $[DA]_{\max}$ varied by striatal sub-region, as shown by the analysis of individual drug treatments (Figs. 4 to 6). However, only vesicular release, but not uptake, exhibited a similar, region-distinct variation. Moreover, while AMPH significantly slowed DA uptake in all treatments, we did not observe a significant effect of striatal sub-region on this inhibition for any drug treatment. Second, this same issue was addressed across treatments in Figure 8. The x-axis clearly shows the variation of treatment effects on the response of $[DA]_{\max}$ to AMPH, ranging from <20 % to >200 % pre-treatment. However, vesicular release better correlated with this variation in $[DA]_{\max}$ than uptake. The poorer correlation with uptake was not due to limited dynamic range, as uptake varied from pre-AMPH baseline levels near 100 % to near 25 % after amphetamine.

The veracity of the kinetic analysis is defended by the demonstration that in both control treatments AMPH decreased DA uptake and increased DA vesicular release in the dorsal and ventral striatum, which we have previously established. AMPH also slowed DA uptake in all treatments, i.e., two controls and three drug treatments, as expected for a DA uptake

inhibitor. Moreover, analysis of the combined pre-treatment data set showed significantly higher DA uptake in the dorsal compared to the ventral striatum, which is also consistent with our previous work (Garris and Wightman 1994; Garris *et al.* 1994; Wu *et al.* 2001a,b). Interestingly, we did not observe greater vesicular DA release and lower evoked DA levels in the dorsal striatum, as shown before, but these differences could be due to the shorter train duration used in the present study (0.4 versus 2.0 s). Longer train durations interrogate different storage pools (Rizzoli and Betz 2005) and allow more time for uptake to exert control over evoked DA levels during the stimulation compared to shorter train durations. This latter point is buttressed by the better correlation between vesicular release and $[DA]_{\max}$ evoked by the 0.4-s, reinforcing-like stimulation than for uptake (Fig. 8).

One important caveat of the experimental design was the differential effects of treatments on pre-AMPH baseline $[DA]_{\max}$ (Fig. 9). By reflecting the status of releasable DA stores, these treatment effects may have altered AMPH responses to some degree. For example, the AMPH-induced increase in $[DA]_{\max}$ after α MpT in the ventral striatum may not be as great as either control, because the releasable pool was reduced by inhibiting dopamine synthesis. This α MpT-induced decrease in the releasable pool was offset by pargyline in the combined drug cocktail, where the response to AMPH was similar to controls. Restoring $[DA]_{\max}$ alone is not sufficient to return the AMPH response to control levels, because AMPH elicited minimal to no change in the dorsal striatum after the cocktail of α MpT and pargyline, suggestive of a different AMPH mechanism in this striatal sub-region. A ceiling effect may conversely explain why the AMPH-induced increase in $[DA]_{\max}$ in the ventral striatum after pargyline was not as great as controls and the combined drug treatment. Indeed, baseline $[DA]_{\max}$ after pargyline in this striatal region was the highest of any treatment. There may thus be a limit as to how much AMPH can increase $[DA]_{\max}$ after pargyline when vesicular release is already activated. Clearly, more work needs to be done to better understand the nuances of these treatment effects.

Mechanism of AMPH Action on Vesicular DA Release

The AMPH-induced increase in vesicular DA release was prevented in the dorsal striatum by the drug cocktail of α MpT and pargyline. This result is consistent with a mechanism of AMPH inhibiting monoamine oxidase (Scorza *et al.* 1997) and/or activating tyrosine hydroxylase (Kuczenski 1975) leading to elevated cytosolic DA levels, greater packaging of DA into vesicles, and ultimately increased quantal size. At this time, it is not possible to determine the respective contributions of DA degradation and synthesis, because both α MpT and pargyline alone largely prevented the AMPH-induced enhancement of vesicular DA release. Nevertheless, our results are consistent with DA degradation and synthesis as the predominant targets for AMPH augmenting vesicular DA release in the dorsal striatum.

In contrast, the AMPH-induced augmentation of vesicular DA release in the ventral striatum was insensitive to the combination of α MpT and pargyline. This result suggests an alternative mechanism to activating DA synthesis and inhibiting DA degradation in this striatal sub-region, perhaps mobilizing the reserve DA pool, as has previously been proposed albeit not proven for cocaine and amfonelic acid in studies with a similar experimental design as used here (Ewing *et al.* 1983; Kuhr *et al.* 1986; Venton *et al.* 2006). Transgenic synapsin-knockout mice show that cocaine-induced increases in vesicular DA release are mediated by synapsin (Venton *et al.* 2006; Kile *et al.* 2010), a protein that segregates vesicles into the reserve pool (Greengard *et al.* 1993; Pieribone *et al.* 1995). It is thus interesting to speculate that AMPH similarly acts on a synapsin-dependent reserve DA pool to enhance vesicular DA release. α -Synuclein, another synaptic protein involved in vesicular storage and trafficking and implicated in methylphenidate-induced up-regulation

of vesicular DA release (Chadchankar *et al.* 2012), should also be considered. Other potential targets for AMPH in the ventral striatum are identified in **INTRODUCTION**.

Although lower doses of AMPH also inhibit DA degradation and activate DA synthesis (Kuczenski 1983), whether they similarly contribute to increases in vesicular DA release in the dorsal striatum as observed here with 10 mg/kg requires further study. Nevertheless, at a minimum, our results demonstrate a robust enhancement of vesicular DA release in the ventral striatum mimicking the actions of cocaine and amfonelic acid, and are relevant to high-dose behavioral effects of AMPH, such as stereotypy and toxic psychosis (Seiden *et al.* 1993). Moreover, because of the controversial nature of AMPH mechanism, our results of augmented vesicular DA release are contrasted with the numerous reports of AMPH depleting vesicular DA stores (*vide infra*).

DAT Inhibitors and Vesicular DA Release

Several DAT inhibitors have been shown to augment vesicular DA release, including cocaine (Jones *et al.* 1995; Lee *et al.* 2001; Venton *et al.* 2006; Oleson *et al.* 2009; Kile *et al.* 2010), methylphenidate (Volz *et al.* 2008; Chadchankar *et al.* 2012), amfonelic acid (Ewing *et al.* 1983), nomifensine (Lee *et al.* 2001), and WIN 35428 (Lee *et al.* 2001). There is now emerging evidence to suggest that AMPH also acts similarly (present study; Ramsson *et al.* 2011b, Daberkow *et al.* 2013). It remains to be determined whether a mechanism of enhancing vesicular DA release generalizes to all DAT inhibitors and to what extent, and the relevance of this mechanism to overall drug effect. Because of its complex actions, including effects on tyrosine hydroxylase, monoamine oxidase, and membrane excitability, AMPH may prove to be more efficacious in enhancing vesicular DA release than psychostimulants predominantly acting via DAT inhibition.

One complication in assessing the hypothesis that DAT inhibitors augment vesicular DA release is that these effects are stimulus dependent and differ between preparations (Ramsson *et al.* 2011a). Indeed, cocaine and amphetamine both robustly inhibit DA uptake and enhance vesicular DA release *in vivo* when evaluated using short-duration pulse trains as we have used here (Venton *et al.* 2006; Oleson *et al.* 2009; Ramsson *et al.* 2011b; Daberkow *et al.* 2013), whereas the predominant effect using pulse trains of intermediate duration is uptake inhibition (May *et al.* 1988; Wu *et al.* 2001a; Ramsson *et al.* 2011a). Moreover, AMPH decreases vesicular DA release in brain slices (Jones *et al.* 1998; Schmitz *et al.* 2001; Patel *et al.* 2003) and single cells (Chen and Ewing 1995; Anderson *et al.* 1998), but only long-duration trains reveal this effect *in vivo* (Ewing *et al.* 1983; Kuhr *et al.* 1985; Kuhr *et al.* 1986). It will thus be necessary to compare DAT inhibitors using uniform experimental conditions that support enhanced vesicular DA release.

Another important issue is the respective contribution of altered DA release versus uptake in mediating the effects of DAT inhibitors on DA function. Uptake is clearly an important site of action, because drug potencies for DAT binding and self-administration correlate well (Ritz *et al.* 1987), and DAT inhibitors can increase striatal levels of extracellular DA in the absence of effects on vesicular DA release (Wu *et al.* 2001a; Ramsson *et al.* 2011a). Although AMPH both robustly inhibits DA uptake and augments vesicular DA release in awake animals, DA release is better correlated with $[DA]_{\max}$ (Daberkow *et al.* 2013). We extend this finding in the present study to canvass a broader range of values to include conditions of disrupted vesicular DA release. Taken together, these results suggest that augmented vesicular DA release plays the more predominant role in mediating the AMPH-induced augmentation of $[DA]_{\max}$ evoked by the reinforcing-like stimulation.

The DAT inhibitors, cocaine (Stuber *et al.* 2005) and nomifensine (Robinson and Wightman 2004), have additionally been shown to augment naturally occurring phasic DA transients.

These sub-second increases in extracellular DA are generated by burst firing, occur spontaneously or in response to rewards and their predictive cues, and are important learning signals in goal-directed behavior (Wanat *et al.* 2009). Their hyper-activation by drugs of abuse is proposed to usurp reward-related circuits during addiction (Hyman 2005). We have also demonstrated an up-regulation of phasic DA transients with AMPH (Ramsson *et al.* 2011b; Daberkow *et al.* 2013). The DA responses evoked by the reinforcing-like stimulation resemble these physiological DA signals in amplitude and dynamics (Robinson and Wightman 2007) and as described above, are enhanced by AMPH to a greater extent by increases in vesicular DA release compared to decreases in DA uptake. It can thus be argued that AMPH-induced increases in vesicular DA release may play a more prominent role in activating phasic DA signaling than decreased DA uptake.

Regional Effects of Abused Drugs

Drugs of abuse elicit regionally distinct actions in the striatum, with primary reinforcing and conditional effects mediated by the ventral portion and instrumental learning and habit formation mediated by the dorsal portion (Everitt and Robbins 2005). Abused drugs, such as cocaine and AMPH, also preferentially increase extracellular DA levels in the ventral versus dorsal striatum (Di and Imperato 1988; Carboni *et al.* 1989; Kuczenski and Segal 1992; Cass *et al.* 1992; Wu *et al.* 2001a; Ramsson *et al.* 2011b). The present findings extend regional effects of addictive drugs in the striatum to distinct mechanisms for AMPH augmenting vesicular DA release. Although the functional significance of our findings is not established, one possibility is that distinct mechanisms confer a differential ability to maintain activated DA signaling. For example, extracellular DA levels electrically evoked by long-duration stimulation are less sensitive to the depleting effects of AMPH in the ventral compared to the dorsal striatum (Ewing *et al.* 1983; Kuhr *et al.* 1985; Kuhr *et al.* 1986; Ramsson *et al.* 2011a). It may thus be that the mechanism by which AMPH increases vesicular DA release in the ventral striatum is better capable of sustaining higher rates of release than in the dorsal striatum, which is more dependent upon DA degradation and synthesis. Regionally distinct effects of AMPH on vesicular DA release may additionally contribute to the preferential increases in extracellular DA levels in the ventral striatum.

Conclusion

We show that AMPH uniquely augments vesicular DA release in the dorsal and ventral striatum and that these effects contribute more to the amplitude of phasic-like DA signals than DA uptake inhibition. The present results thus advance our understanding of the complex cellular mechanisms targeted by this psychostimulant and further highlight vesicular DA release as an important site of action for DAT inhibitors. Future studies should explore the role of DA synthesis, degradation and storage pools in mediating the effects of AMPH on behaviorally relevant DA signals under physiological conditions and further characterize the effects of MAO inhibition on DA signaling in striatal sub-regions.

Acknowledgments

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Abbreviations

AMPH	amphetamine
α MpT	alpha-methyl-para-tyrosine

DA	dopamine
DAT	dopamine transporter

REFERENCES

- Aceto MD, Botton I, Martin R, Levitt M, Bentley HC, Speight PT. Pharmacologic properties and mechanism of action of amfonelic acid. *Eur. J. Pharmacol.* 1970; 10:344–354. [PubMed: 4393073]
- Anderson BB, Chen G, Gutman DA, Ewing AG. Dopamine levels of two classes of vesicles are differentially depleted by amphetamine. *Brain Res.* 1998; 788:294–301. [PubMed: 9555063]
- Brown JM, Hanson GR, Fleckenstein AE. Cocaine-induced increases in vesicular dopamine uptake: role of dopamine receptors. *J. Pharmacol. Exp. Ther.* 2001; 298:1150–1153. [PubMed: 11504813]
- Carboni E, Imperato A, Perezzi L, Di Chiara G. Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. *Neuroscience.* 1989; 28:653–661. [PubMed: 2710338]
- Cass WA, Gerhardt GA, Mayfield RD, Curella P, Zahniser NR. Differences in dopamine clearance and diffusion in rat striatum and nucleus accumbens following systemic cocaine administration. *J. Neurochem.* 1992; 59:259–266. [PubMed: 1613502]
- Chadchankar H, Ihalaainen J, Tanila H, Yavich L. Methylphenidate modifies overflow and presynaptic compartmentalization of dopamine via an alpha-synuclein-dependent mechanism. *J. Pharmacol. Exp. Ther.* 2012; 341:484–492. [PubMed: 22344407]
- Cheer JF, Aragona BJ, Heien ML, Seipel AT, Carelli RM, Wightman RM. Coordinated accumbal dopamine release and neural activity drive goal-directed behavior. *Neuron.* 2007; 54:237–244. [PubMed: 17442245]
- Chen G, Ewing AG. Multiple classes of catecholamine vesicles observed during exocytosis from the Planorbis cell body. *Brain Res.* 1995; 701:167–174. [PubMed: 8925280]
- Daberkow DP, Brown HD, Bunner KD, Kraniotis SA, Doellman MA, Ragozino ME, Garris PA, Roitman MF. Amphetamine paradoxically augments exocytotic dopamine release and phasic dopamine signals. *J. Neurosci.* 2013; 33:452–463. [PubMed: 23303926]
- Di CG, Imperato A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. Natl. Acad. Sci. U. S. A.* 1988; 85:5274–5278. [PubMed: 2899326]
- Everitt BJ, Robbins TW. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat. Neurosci.* 2005; 8:1481–1489. [PubMed: 16251991]
- Ewing AG, Bigelow JC, Wightman RM. Direct in vivo monitoring of dopamine released from two striatal compartments in the rat. *Science.* 1983; 221:169–171. [PubMed: 6857277]
- Fleckenstein AE, Haughey HM, Metzger RR, Kokoshka JM, Riddle EL, Hanson JE, Gibb JW, Hanson GR. Differential effects of psychostimulants and related agents on dopaminergic and serotonergic transporter function. *Eur. J. Pharmacol.* 1999; 382:45–49. [PubMed: 10556503]
- Fleckenstein AE, Volz TJ, Riddle EL, Gibb JW, Hanson GR. New insights into the mechanism of action of amphetamines. *Annu. Rev. Pharmacol. Toxicol.* 2007; 47:681–698. [PubMed: 17209801]
- Garris PA, Wightman RM. Different kinetics govern dopaminergic transmission in the amygdala, prefrontal cortex, and striatum: an in vivo voltammetric study. *J. Neurosci.* 1994; 14:442–450. [PubMed: 8283249]
- Garris PA, Ciolkowski EL, Wightman RM. Heterogeneity of evoked dopamine overflow within the striatal and striatoamygdaloid regions. *Neuroscience.* 1994; 59:417–427. [PubMed: 8008199]
- Garris PA, Christensen JR, Rebec GV, Wightman RM. Real-time measurement of electrically evoked extracellular dopamine in the striatum of freely moving rats. *J. Neurochem.* 1997; 68:152–161. [PubMed: 8978721]
- Greengard P, Valtorta F, Czernik AJ, Benfenati F. Synaptic vesicle phosphoproteins and regulation of synaptic function. *Science.* 1993; 259:780–785. [PubMed: 8430330]
- Heal DJ, Cheetham SC, Smith SL. The neuropharmacology of ADHD drugs in vivo: insights on efficacy and safety. *Neuropharmacology.* 2009; 57:608–618. [PubMed: 19761781]

- Hyman SE. Addiction: a disease of learning and memory. *Am. J. Psychiatry*. 2005; 162:1414–1422. [PubMed: 16055762]
- Ingram SL, Prasad BM, Amara SG. Dopamine transporter-mediated conductances increase excitability of midbrain dopamine neurons. *Nat. Neurosci*. 2002; 5:971–978. [PubMed: 12352983]
- Jones SR, Gainetdinov RR, Wightman RM, Caron MG. Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. *J. Neurosci*. 1998; 18:1979–1986. [PubMed: 9482784]
- Jones SR, Garris PA, Wightman RM. Different effects of cocaine and nomifensine on dopamine uptake in the caudate-putamen and nucleus accumbens. *J. Pharmacol. Exp. Ther*. 1995; 274:396–403. [PubMed: 7616424]
- Kile BM, Guillot TS, Venton BJ, Wetsel WC, Augustine GJ, Wightman RM. Synapsins differentially control dopamine and serotonin release. *J. Neurosci*. 2010; 30:9762–9770. [PubMed: 20660258]
- Kuczenski R. Effects of catecholamine releasing agents on synaptosomal dopamine biosynthesis: multiple pools of dopamine or multiple forms of tyrosine hydroxylase. *Neuropharmacology*. 1975; 14:1–10. [PubMed: 239360]
- Kuczenski, R. Biochemical actions of amphetamine and other stimulants. In: Creese, I., editor. *Stimulants: neurochemical, behavioral and clinical perspectives*. New York: Raven Press; 1983. p. 31–61.
- Kuczenski R, Segal DS. Differential effects of amphetamine and dopamine uptake blockers (cocaine, nomifensine) on caudate and accumbens dialysate dopamine and 3-methoxytyramine. *J. Pharmacol. Exp. Ther*. 1992; 262:1085–1094. [PubMed: 1527715]
- Kuhr WG, Bigelow JC, Wightman RM. In vivo comparison of the regulation of releasable dopamine in the caudate nucleus and the nucleus accumbens of the rat brain. *J. Neurosci*. 1986; 6:974–982. [PubMed: 3486259]
- Kuhr WG, Ewing AG, Near JA, Wightman RM. Amphetamine attenuates the stimulated release of dopamine in vivo. *J. Pharmacol. Exp. Ther*. 1985; 232:388–394. [PubMed: 3968641]
- Lee TH, Balu R, Davidson C, Ellinwood EH. Differential time-course profiles of dopamine release and uptake changes induced by three dopamine uptake inhibitors. *Synapse*. 2001; 41:301–310. [PubMed: 11494401]
- Logman MJ, Budygin EA, Gainetdinov RR, Wightman RM. Quantitation of in vivo measurements with carbon fiber microelectrodes. *J. Neurosci. Methods*. 2000; 95:95–102. [PubMed: 10752479]
- May LJ, Kuhr WG, Wightman RM. Differentiation of dopamine overflow and uptake processes in the extracellular fluid of the rat caudate nucleus with fast-scan in vivo voltammetry. *J. Neurochem*. 1988; 51:1060–1069. [PubMed: 2971098]
- May LJ, Wightman RM. Heterogeneity of stimulated dopamine overflow within rat striatum as observed with in vivo voltammetry. *Brain Res*. 1989; 487:311–320. [PubMed: 2786444]
- Michael D, Travis ER, Wightman RM. Color images for fast-scan CV measurements in biological systems. *Anal Chem*. 1998; 70:586A–592A.
- Mundorf ML, Hochstetler SE, Wightman RM. Amine weak bases disrupt vesicular storage and promote exocytosis in chromaffin cells. *J. Neurochem*. 1999; 73:2397–2405. [PubMed: 10582599]
- Oleson EB, Salek J, Bonin KD, Jones SR, Budygin EA. Real-time voltammetric detection of cocaine-induced dopamine changes in the striatum of freely moving mice. *Neurosci. Lett*. 2009; 467:144–146. [PubMed: 19822192]
- Patel J, Mooslehner KA, Chan PM, Emson PC, Stamford JA. Presynaptic control of striatal dopamine neurotransmission in adult vesicular monoamine transporter 2 (VMAT2) mutant mice. *J. Neurochem*. 2003; 85:898–910. [PubMed: 12716422]
- Paxinos, G.; Watson, C. *The rat brain in stereotaxic coordinates*. New York: Academic Press; 1986.
- Peacock J, Benca RM. Narcolepsy: clinical features, co-morbidities & treatment. *Indian J. Med. Res*. 2010; 131:338–349. [PubMed: 20308759]
- Pieribone VA, Shupliakov O, Brodin L, Hilfiker-Rothenfluh S, Czernik AJ, Greengard P. Distinct pools of synaptic vesicles in neurotransmitter release. *Nature*. 1995; 375:493–497. [PubMed: 7777058]
- Ramsson ES, Covey DP, Daberkow DP, Litherland MT, Juliano SA, Garris PA. Amphetamine augments action potential-dependent dopaminergic signaling in the striatum in vivo. *J. Neurochem*. 2011a; 117:937–948. [PubMed: 21443523]

- Ramsson ES, Howard CD, Covey DP, Garris PA. High doses of amphetamine augment, rather than disrupt, exocytotic dopamine release in the dorsal and ventral striatum of the anesthetized rat. *J. Neurochem.* 2011b; 119:1162–1172. [PubMed: 21806614]
- Riddle EL, Hanson GR, Fleckenstein AE. Therapeutic doses of amphetamine and methylphenidate selectively redistribute the vesicular monoamine transporter-2. *Eur. J. Pharmacol.* 2007; 571:25–28. [PubMed: 17618619]
- Riddle EL, Topham MK, Haycock JW, Hanson GR, Fleckenstein AE. Differential trafficking of the vesicular monoamine transporter-2 by methamphetamine and cocaine. *Eur. J. Pharmacol.* 2002; 449:71–74. [PubMed: 12163108]
- Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science.* 1987; 237:1219–1223. [PubMed: 2820058]
- Rizzoli SO, Betz WJ. Synaptic vesicle pools. *Nat. Rev. Neurosci.* 2005; 6:57–69. [PubMed: 15611727]
- Robinson DL, Wightman RM. Nomifensine amplifies subsecond dopamine signals in the ventral striatum of freely-moving rats. *J. Neurochem.* 2004; 90:894–903. [PubMed: 15287895]
- Robinson, DL.; Wightman, RM. Rapid dopamine release in freely moving rats. In: Michael, AC.; Borland, LM., editors. *Electrochemical Methods for Neuroscience*. Boca Raton: CRC Press; 2007. p. 17-34.
- Schmitz Y, Lee CJ, Schmauss C, Gonon F, Sulzer D. Amphetamine distorts stimulation-dependent dopamine overflow: effects on D2 autoreceptors, transporters, and synaptic vesicle stores. *J. Neurosci.* 2001; 21:5916–5924. [PubMed: 11487614]
- Scorza MC, Carrau C, Silveira R, Zapata-Torres G, Cassels BK, Reyes-Parada M. Monoamine oxidase inhibitory properties of some methoxylated and alkylthio amphetamine derivatives: structure-activity relationships. *Biochem. Pharmacol.* 1997; 54:1361–1369. [PubMed: 9393679]
- Seiden LS, Sabol KE, Ricaurte GA. Amphetamine: effects on catecholamine systems and behavior. *Annu. Rev. Pharmacol. Toxicol.* 1993; 33:639–677. [PubMed: 8494354]
- Shore PA. Actions of amfonelic acid and other non-amphetamine stimulants on the dopamine neuron. *J. Pharm. Pharmacol.* 1976; 28:855–857. [PubMed: 11304]
- Stuber GD, Wightman RM, Carelli RM. Extinction of cocaine self-administration reveals functionally and temporally distinct dopaminergic signals in the nucleus accumbens. *Neuron.* 2005; 46:661–669. [PubMed: 15944133]
- Sulzer D. How addictive drugs disrupt presynaptic dopamine neurotransmission. *Neuron.* 2011; 69:628–649. [PubMed: 21338876]
- Venton BJ, Seipel AT, Phillips PE, Wetsel WC, Gitler D, Greengard P, Augustine GJ, Wightman RM. Cocaine increases dopamine release by mobilization of a synapsin-dependent reserve pool. *J. Neurosci.* 2006; 26:3206–3209. [PubMed: 16554471]
- Volz TJ, Farnsworth SJ, King JL, Riddle EL, Hanson GR, Fleckenstein AE. Methylphenidate administration alters vesicular monoamine transporter-2 function in cytoplasmic and membrane-associated vesicles. *J. Pharmacol. Exp. Ther.* 2007; 323:738–745. [PubMed: 17693585]
- Volz TJ, Farnsworth SJ, Rowley SD, Hanson GR, Fleckenstein AE. Methylphenidate-induced increases in vesicular dopamine sequestration and dopamine release in the striatum: the role of muscarinic and dopamine D2 receptors. *J. Pharmacol. Exp. Ther.* 2008; 327:161–167. [PubMed: 18591219]
- Wanat MJ, Willuhn I, Clark JJ, Phillips PE. Phasic dopamine release in appetitive behaviors and drug addiction. *Curr. Drug Abuse Rev.* 2009; 2:195–213. [PubMed: 19630749]
- Wightman RM, Amatore C, Engstrom RC, Hale PD, Kristensen EW, Kuhr WG, May LJ. Real-time characterization of dopamine overflow and uptake in the rat striatum. *Neuroscience.* 1988; 25:513–523. [PubMed: 3399057]
- Wu Q, Reith ME, Kuhar MJ, Carroll FI, Garris PA. Preferential increases in nucleus accumbens dopamine after systemic cocaine administration are caused by unique characteristics of dopamine neurotransmission. *J. Neurosci.* 2001a; 21:6338–6347. [PubMed: 11487657]
- Wu Q, Reith ME, Wightman RM, Kawagoe KT, Garris PA. Determination of release and uptake parameters from electrically evoked dopamine dynamics measured by real-time voltammetry. *J. Neurosci Methods.* 2001b; 112:119–133. [PubMed: 11716947]

Figure 1. Experimental design
See **MATERIALS and METHODS** for details.

Figure 2. Stimulation-only control

Individual recordings evoked by the reinforcing-like stimulation are shown in **Panel A** for the dorsal (top) and ventral (bottom) striatum. Time -2 min (black) was collected during the pre-treatment baseline period, time 118 min (red) was collected during the post-treatment baseline period, and time 138 min (blue) was collected during the post-treatment, AMPH-test period. The short line underneath the recordings demarcates the stimulus pulse train. Averaged results for $[DA]_{max}$ are shown in **Panel B**. Green symbols and arrow demarcate the time of AMPH injection. For the pre- and post-treatment periods, data were calculated as a percent of the average $[DA]_{max}$ evoked by the reinforcing-like stimulation collected during the pre-treatment period; for the treatment period, data were calculated as a percent of the $[DA]_{max}$ evoked by the first depleting stimulation (% pre-treatment). Data for statistical analysis are shown in **Panel C** for $[DA]_{max}$ (left), vesicular DA release or $[DA]_p$ (middle), and DA uptake or k (right). Time 118 min was collected immediately before AMPH administration, time 128 min was collected 10 min after AMPH administration and time 138 min was collected 20 min after AMPH administration. Data were calculated as a percent of the average responses collected during the pre-treatment period (% pre-treatment). In **Panels B and C**, data are expressed as mean $\pm$ SEM.

Figure 3. Control treatment without the depleting stimulation

See legend to Figure 2 for details, with the exception that no depleting stimulations were applied and no drug was injected during the treatment period.

Figure 4. Pharmacological treatment with αMPT

See legend to Figure 2 for details, with the exception that αMPT was injected during the treatment period (blue symbols and left arrow).

Figure 5. Pharmacological treatment with pargyline

See legend to Figure 2 for details, with the exception that pargyline was injected during the treatment period (blue symbols and left arrow).

Figure 6. Pharmacological treatment with α MpT and pargyline

See legend to Figure 2 for details, with the exception that α MpT and pargyline were injected during the treatment period (blue symbols and left arrow).

Figure 7. Effects of AMPH on $[DA]_{\max}$ across all treatment groups

Panels A and B show $[DA]_{\max}$ evoked by the reinforcing-like stimulation during the post-treatment period and collected in the dorsal and ventral striatum, respectively. Data are from Figures 2 to 6 and were normalized by subtracting the value at time 0 min (equivalent of time 118 min) from values at all of the time points in each animal and then averaging across groups. “ Δ % pre-AMPH” thus represents the absolute percent increase in $[DA]_{\max}$ during the post-treatment period. The black square demarcates the time of AMPH injection. Data are expressed as the mean $\pm$ SEM. Treatment key: no-stimulation control - no-depleting stimulation control; stimulation control - depleting stimulation-only control; α MpT - depleting stimulation with α MpT; pargyline - depleting stimulation with pargyline; α MpT, pargyline - depleting stimulating with α MpT and pargyline. *, significantly different from the pooled control group at $p < 0.05$. NS, no significant main effect of treatment.

Figure 8. Relationships between vesicular DA release (A) or DA uptake (B) and $[DA]_{\max}$
Data are from **Panel C** of Figures 2 to 6. All treatment groups in both dorsal and ventral striata were combined. The solid line is the best-fit line for linear regression. r , regression coefficient.

Figure 9. Treatments effects on pre-AMPH baseline $[DA]_{max}$

Data are from Figures 2 to 6 and show $[DA]_{max}$ evoked by the reinforcing-like stimulation and collected at time 118 min during the post-treatment period in the dorsal and ventral striata. This time point, identified as time 0 min, is immediately before AMPH administration. Data are expressed as the mean $\pm$ SEM. Abbreviations: – stim, no-depleting stimulation control; + stim, depleting stimulation-only control; αMT, αMpT; P, pargyline; αMT, P; αMpT and pargyline.