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ORIGINAL ARTICLE

Amphetamine potency varies with dopamine uptake rate across striatal subregions

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Abstract

Amphetamine is a central nervous system psychostimulant with a high potential for abuse. Recent literature has shown that genetic and drug-induced elevations in dopamine transporter (DAT) expression augment the neurochemical and behavioral potency of psychostimulant releasers. However, it remains to be determined if the well-documented differences in DAT levels across striatal regions drive regionally distinct amphetamine effects within individuals. DAT levels and dopamine uptake rates have been shown to follow a gradient in the striatum, with the highest levels in the dorsal regions and lowest levels in the nucleus accumbens shell; thus, we hypothesized that amphetamine potency would follow this gradient. Using fast scan cyclic voltammetry in mouse brain slices, we examined DAT inhibition and changes in exocytotic dopamine release by amphetamine across four striatal regions (dorsal and ventral caudate-putamen, nucleus accumbens core and shell). Consistent with our hypothesis, amphetamine effects at the DAT and on release decreased across regions from dorsal to ventral, and both measures of potency were highly correlated with dopamine uptake rates. Separate striatal subregions are involved in different aspects of motivated behaviors, such as goal-directed and habitual behaviors, that become dysregulated by drug abuse, making it critically important to understand regional differences in drug potencies.

Keywords: caudate, dopamine transporter, mouse, nucleus accumbens, releaser, voltammetry.

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Amphetamine (AMPH), a commonly prescribed and highly addictive psychostimulant, has been characterized as a dopamine releaser that alters dopamine neurotransmission primarily through interactions with the dopamine transporter (DAT). AMPH produces inhibition of dopamine uptake as well as DAT-mediated reverse transport, resulting in robust increases in extracellular dopamine, most notably in the terminal regions of dopaminergic projections from the ventral midbrain, such as the dorsal and ventral striatum. AMPH-induced elevations in the striatal dopamine have been shown to be critical for its reinforcing and rewarding effects (Fleckenstein *et al.* 2007; Sulzer 2011). Recent results have indicated that the actions of AMPH on dopamine neurotransmission are altered by DAT expression, with increased DAT levels and dopamine uptake rates resulting in increased AMPH potency (Salahpour *et al.* 2008; Calipari *et al.* 2013). Previous reports linking DAT levels to AMPH potency used genetic manipulations to alter DAT levels; however, the results suggest that natural variations in DAT expression across striatal subregions may also affect AMPH potency.

While the striatum is integrally involved in the reinforcing effects of drugs of abuse, including AMPH, it is a heterogeneous area with specific sub-regions that mediate different aspects of drug reinforcement (Everitt and Robbins 2013). For example, the nucleus accumbens (NAc) mediates the subjective and discriminative effects of psychostimulants as well as goal-directed responding on a reward paired operanda (Roberts *et al.* 1980; Zito *et al.* 1985; Drevets *et al.* 2001), while the dorsal caudate-putamen (CPu) has been shown to be involved in habit learning and responding for cues previously paired with drug delivery (Ito *et al.* 2002;

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Abbreviations used: AMPH, amphetamine; CPu, caudate-putamen; DAT, dopamine transporter; FSCV, fast scan cyclic voltammetry; NAc, nucleus accumbens.

Gabriele and See 2011). Both habitual and goal-directed behaviors play critical roles in the addiction process, and the striatal regions that control these behaviors have been shown to be differentially dysregulated by drug abuse. Multiple studies have demonstrated that AMPH-induced dopamine overflow is greatest in the dorsal CPu as compared to more ventral regions of the striatum (Hernandez *et al.* 1987; Pehek *et al.* 1990; Robinson and Camp 1990; Kuczenski *et al.* 1991; Kuczenski and Segal 1992); however, some groups have shown the converse (Sharp *et al.* 1987; Di Chiara and Imperato 1988). While *in vivo* studies are able to assess a number of factors that may be contributing to the differential effects of AMPH across anatomical regions, including divergent afferent inputs and local regulation of dopamine release by interneurons, we sought to examine how regional variations in DAT function, may influence AMPH potency.

Here we used fast scan cyclic voltammetry (FSCV) in brain slices to assess AMPH potency in four regions spanning the CPu and NAc. Previous works show that DAT levels and dopamine uptake rates are highest in dorsal regions and decrease incrementally from dorsal to ventral (Coulter *et al.* 1996; Yeghiayan *et al.* 1997; South and Huang 2008; Calipari *et al.* 2012) and that genetic augmentation of DAT levels increases psychostimulant releaser potency (Calipari *et al.* 2013); therefore, we hypothesized that the effects of AMPH on dopamine neurotransmission would be more potent in dorsal regions. Given the distinct contributions of striatal sub-regions to addictive behaviors, it is critically important to determine if these areas are differentially affected by AMPH, and if so, the mechanism underlying the variations in potency.

Materials and methods

Animals

Male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME, USA) were maintained on a 12 : 12 h light–dark cycle (6:00 AM lights on; 6:00 PM lights off) with food and water *ad libitum.* All animals were maintained in accordance with the National Institutes of Health guidelines in Association for Assessment and Accreditation of Laboratory Animal Care accredited facilities. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Wake Forest School of Medicine.

In vitro voltammetry

FSCV was used to characterize presynaptic dopamine signaling and AMPH potency in the dorsal CPu, ventral CPu, NAc core, and NAc shell. A vibrating tissue slicer was used to prepare 400 μm thick coronal brain sections. The tissue was immersed in oxygenated artificial cerebrospinal fluid containing (in mM): NaCl (126), KCl (2.5), NaH_2PO_4 (1.2), $CaCl_2$ (2.4), $MgCl_2$ (1.2), $NaHCO_3$ (25), glucose (11), L-ascorbic acid (0.4), and pH was adjusted to 7.4. Once sliced, the tissue was transferred to testing chambers containing bath artificial cerebrospinal fluid (32°C), which flowed at 1 mL/min. A carbon fiber microelectrode (100–200 μM length, 7 μM radius) and bipolar stimulating electrode were placed in close proximity in the region of interest. Extracellular dopamine was measured by applying a triangular waveform (−0.4 to +1.2 to −0.4V vs. Ag/AgCl, 400 V/s) to the recording electrode every 100 ms.

Dopamine release was evoked by a single electrical pulse (300 μA, 4 ms, monophasic) applied to the tissue every 3 min until a stable baseline was established (three collections within 10% variability). After pre-drug measures were taken, increasing AMPH concentrations (10 nM, 100 nM, 300 nM, 1 μM, 3 μM, and 10 μM) were cumulatively bath applied to each slice. Stimulations were repeated until evoked dopamine levels reached stability at each concentration (approximately 30 min).

Data analysis

For analysis of FSCV data, Demon Voltammetry and Analysis software was used (Yorgason *et al.* 2011). Michaelis–Menten modeling parameters were used to determine the maximal rate of dopamine uptake and AMPH-induced uptake inhibition (Wightman *et al.* 1998). Michaelis–Menten modeling provides parameters that describe the amount of dopamine released following electrical stimulation, the maximal rate of dopamine uptake (V_{max}), and alterations in the ability of dopamine to bind to the DAT, or apparent K_m. For pre-drug modeling, we followed standard voltammetric modeling procedures by setting the baseline K_m parameter to 160 nM based on the affinity of dopamine for the DAT, whereas V_{max} values were allowed to vary as the pre-drug measure of the rate of dopamine uptake. Following drug application, apparent K_m was allowed to vary to account for changes in drug-induced dopamine uptake inhibition while the respective V_{max} value determined for that subject at baseline was held constant. The apparent K_m parameter models the amount of dopamine uptake inhibition following a particular concentration of drug.

Recording electrodes were calibrated by recording responses (in electrical current; nA) to a known concentration of dopamine (3 μM) using a flow-injection system. Calibrations were used to convert electrical current to dopamine concentration.

K_i values

As described previously (Jones *et al.* 1995), inhibition constants (K_i) were calculated by the equation: $[(K_m)/(S)]$, where K_m is the K_m of dopamine for the dopamine transporter, or 1.6 μM, and S is the slope of the linear concentration–response regression for amphetamine. The K_i, reported in μM and is a measure of the AMPH concentration that is necessary to decrease the rate of dopamine-DAT interactions to 50% of their uninhibited rate.

Statistics

Graph Pad Prism (version 5; La Jolla, CA, USA) was used to statistically analyze data sets and create graphs. Baseline dopamine kinetics and K_i values were compared with a one-way analysis of variance (ANOVA) to test for differences across regions. When main effects were obtained differences between groups were tested using Dunnett's *post hoc* test. Uptake inhibition and release data were subject to a repeated measures two-way ANOVA with AMPH concentration and region as the factors. When main effects were obtained differences between groups were tested using Bonferroni's *post hoc* test. Pearson's correlation coefficients were used to

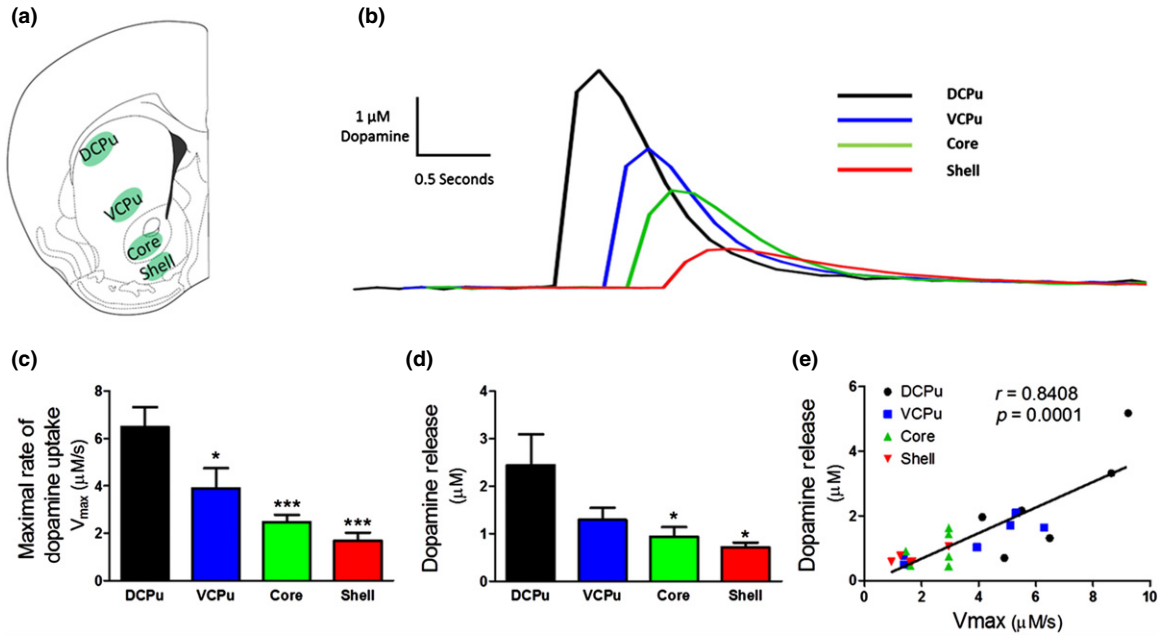

Fig. 1 Assessing subsecond dopamine release and uptake kinetics across striatal subregions. (a) Location of electrode placements for voltammetric assessment of dopamine kinetics and amphetamine potency. Measurements were taken from four striatal subregions. Dorsal caudate-putamen (DCPu); ventral caudate-putamen (VCPu); nucleus accumbens core (Core); nucleus accumbens shell (Shell). (b) Representative traces illustrating that both dopamine release and uptake decrease across regions from dorsal to ventral. (c) Group data showing maximal rate of dopamine uptake are highest in the dorsal caudate-putamen as compared to other striatal regions. (d) Group data demonstrating that dopamine release is highest in the dorsal CPu. (e) Correlation showing that dopamine release and uptake increase from ventral to dorsal across striatal subregions in a similar manner. *$p < 0.05$, ***$p < 0.001$ as compared to dorsal caudate-putamen.

measure the strength of correlation between V_{max} and AMPH potency. All p values of < 0.05 were considered to be statistically significant. A within-subjects design was used whereby measurements were taken from each region in each of six animals. Thus, all groups consist of six measures, except for the NAc shell which has an n of 5 because of equipment failure during one of the voltammetric recordings.

Results

Dopamine release and uptake decreased across regions from dorsal to ventral

Subsecond dopamine kinetics were assessed in the dorsal CPu, ventral CPu, NAc core, and NAc shell (Fig. 1a). Consistent with previous results (Calipari *et al.* 2012), we found that dopamine uptake was highest in the dorsal CPu (Fig. 1c): one-way ANOVA ($F_{3,19} = 9.988$, $p = 0.0004$, $n = 6$), Dunnett's post-test: dorsal CPu versus ventral CPu $p < 0.05$, dorsal CPu versus NAc core $p < 0.001$, dorsal CPu versus NAc shell $p < 0.001$. Dopamine release was also highest in more dorsal regions (Fig. 1d): one-way ANOVA ($F_{3,19} = 3.977$, $p = 0.0235$), Dunnett's post-test: dorsal CPu versus ventral CPu $p > 0.05$, dorsal CPu versus NAc core $p < 0.05$, dorsal CPu versus NAc shell $p < 0.05$. Notably, there was no difference in dopamine release between the dorsal and ventral CPu. To determine if dopamine release and uptake decreased across striatal subregions in a similar manner, we correlated dopamine release and uptake. We found a strong positive correlation between dopamine release and uptake (Fig. 1e): correlation, $r = 0.8408$, $p = 0.0001$; linear regression, $\beta = 1.824 \pm 0.2563$.

The ability of AMPH to inhibit dopamine uptake decreased across regions from dorsal to ventral

Following the assessment of dopamine release and uptake kinetics, we sought to determine the regional differences in the ability of AMPH to inhibit the DAT. Based on previous work demonstrating that releaser potency is increased in animals with genetic over-expression of the DAT (Calipari *et al.* 2013), we hypothesized that AMPH inhibition of the DAT would be higher in brain regions with the highest uptake rates (i.e., the dorsal CPu) as compared to regions with lower uptake rates (i.e., the NAc shell). Following bath application of AMPH we found that, indeed, AMPH-induced increases in apparent K_m for dopamine uptake was highest in the dorsal CPu (Fig. 2a): repeated measures two-way ANOVA (AMPH concentration × region, $n = 6$), AMPH concentration ($F_{5,19} = 3.977$, $p = 0.0235$), region ($F_{3,19} = 13.54$, $p = 0.0001$), interaction ($F_{15,95} = 8.409$, $p = 0.0001$),

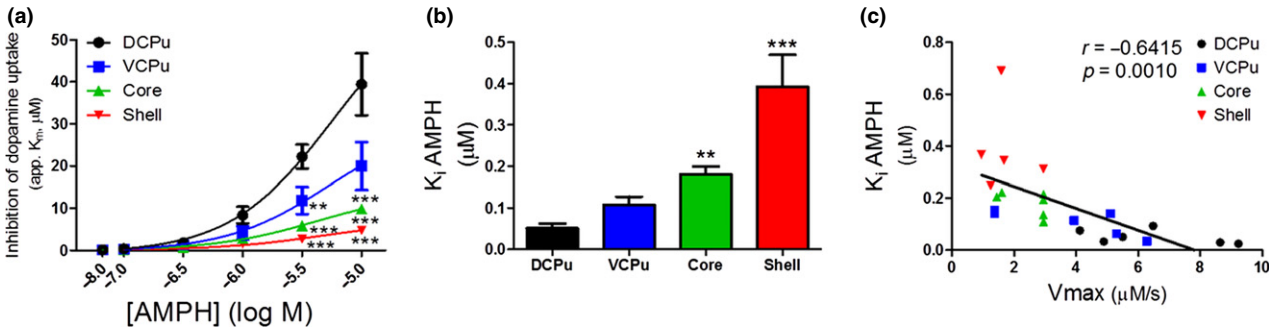

Fig. 2 Inhibition of dopamine uptake (apparent K_m) by amphetamine (AMPH) differs across striatal regions. (a) The ability of AMPH to inhibit dopamine uptake differs across regions from ventral to dorsal. (b) K_i, a measurement of the concentration at which AMPH exerts 50% inhibition, was used to determine AMPH potency across regions. K_i increased across regions from dorsal to ventral, indicating that potency is highest in dorsal regions. (c) K_i and dopamine uptake rates are negatively correlated. DCPu, dorsal caudate-putamen; VCPu, ventral caudate-putamen; Core, nucleus accumbens core; Shell, nucleus accumbens shell. $^{**}p < 0.001$, $^{***}p < 0.001$ as compared to dorsal caudate-putamen.

Bonferroni's post-test: dorsal CPu versus ventral CPu (3 μM) $p < 0.01$, dorsal CPu versus NAc core (3 μM) $p < 0.001$, dorsal CPu versus NAc shell (3 μM) $p < 0.001$, dorsal CPu versus ventral CPu (10 μM) $p < 0.001$, dorsal CPu versus NAc core (10 μM) $p < 0.001$, dorsal CPu versus NAc shell (10 μM) $p < 0.001$.

We then determined the K_i of AMPH, the concentration of drug that results in a 50% inhibition of dopamine uptake, across regions. A decrease in K_i is indicative of an increase in potency. We found that K_i was lowest in the dorsal CPu and increased across regions from dorsal to ventral (Fig. 2b): one-way ANOVA ($F_{3,19} = 15.43$, $p = 0.0007$), Dunnett's post-test: dorsal CPu versus ventral CPu $p > 0.05$, dorsal CPu versus NAc core $p < 0.01$, dorsal CPu versus NAc shell $p < 0.001$.

AMPH inhibition of the DAT was correlated with dopamine uptake rates

V_{max}, a measure of dopamine uptake, allows for a within-subject determination of the relationship between DAT function and drug potencies (Ferris *et al.* 2013). To determine if differences in the ability of AMPH to inhibit the DAT were related to differences in DAT function, we correlated V_{max} with the K_i of AMPH. We found a strong negative correlation between V_{max} and K_i (Fig. 2c): correlation, $r = -0.6415$, $p = 0.0010$; linear regression, $\beta = -0.04075 \pm 0.01063$.

The ability of AMPH to decrease exocytotic dopamine release was highest in the dorsal CPu and lowest in the NAc shell

AMPH has been shown previously to mobilize dopamine from vesicles, thus depleting releasable dopamine pools and leading to decreased evoked dopamine release at high concentrations (Sulzer *et al.* 1990; Sulzer and Rayport 1993; Jones *et al.* 1998, 1999; John and Jones 2007). Conversely, at low concentrations, AMPH has been shown to increase evoked dopamine levels through its actions as a competitive uptake inhibitor (Ramsson *et al.* 2011a,b; Siciliano *et al.* 2014). By measuring electrically-evoked dopamine release we can examine changes in exocytotic release independently of AMPH-induced reverse transport. Here we show that AMPH effects on exocytotic dopamine release differ across regions (Fig. 3a): repeated measures two-way ANOVA (AMPH concentration × region, $n = 6$), AMPH concentration ($F_{5,19} = 27.01$, $p = 0.0001$), region ($F_{3,19} = 2.520$, $p = 0.0887$), interaction ($F_{15,95} = 2.318$, $p = 0.0073$), Bonferroni's post-test: dorsal CPu versus NAc core (10 nM) $p < 0.05$, dorsal CPu versus NAc shell (10 nM) $p < 0.01$, and dorsal CPu versus NAc shell (100 nM) $p < 0.05$.

When data were expressed as a percent change from baseline, AMPH effects on exocytotic release differed across regions; however, post-test analysis did not reveal significance (Fig. 3b): repeated measures two-way ANOVA (AMPH concentration × region), AMPH concentration ($F_{6,19} = 28.43$, $p = 0.0001$), region ($F_{3,19} = 30.99$, $p = 0.0513$), and interaction ($F_{15,95} = 2.318$, $p = 0.0073$). We then determined the IC_{50} of AMPH for exocytotic dopamine release and found that the IC_{50} was lowest in the dorsal CPu and highest in the NAc shell (Fig. 3c): one-way ANOVA ($F_{3,19} = 3.466$, $p = 0.0367$), Dunnett's post-test: dorsal CPu versus ventral CPu $p > 0.05$, dorsal CPu versus NAc core $p > 0.05$, and dorsal CPu versus NAc shell $p < 0.05$.

AMPH effects on exocytotic dopamine release were correlated with dopamine uptake

To determine if AMPH effects on exocytotic dopamine release are a function of uptake rates, we correlated V_{max} with the IC_{50} (the AMPH concentration required to reduce exocytotic dopamine release by 50%) for AMPH-induced inhibition of exocytotic dopamine release and found that as

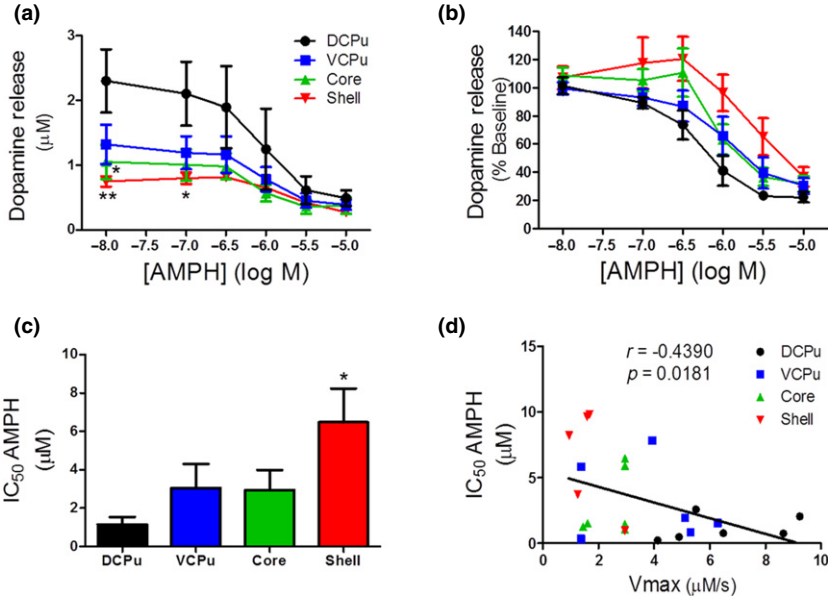

Fig. 3 Amphetamine (AMPH) effects on dopamine release across striatal subregions. (a) AMPH effects on exocytotic dopamine release. (b) The effects of AMPH on dopamine release expressed as a percent change from pre-drug. (c) The IC_{50} of AMPH on exocytotic dopamine release is higher in the shell as compared to the dorsal caudate-putamen. (d) The IC_{50} of AMPH on exocytotic dopamine release and dopamine uptake rates are correlated, indicating that increased dopamine transporter levels lead to greater AMPH-induced depletion of the readily releasable dopamine pool. DCPu, dorsal caudate-putamen; VCPu, ventral caudate-putamen; Core, nucleus accumbens core; Shell, nucleus accumbens shell. $^*p < 0.05$ as compared to dorsal CPu.

V_{max} increased, IC_{50} was decreased (Fig. 3d): correlation, $r = -0.4390$, $p = 0.0181$; linear regression, $\beta = -0.5892 \pm 0.2632$.

Discussion

Here, we show that the magnitude of AMPH effects on exocytotic dopamine release and uptake is positively correlated with maximal dopamine uptake rate, which decreased in a graded manner from dorsal to ventral across the striatum. It has been well documented that DAT protein levels follow a similar gradient (Coulter *et al.* 1996; Yeghiayan *et al.* 1997; South and Huang 2008). Previous work has shown that increasing DAT expression through genetic or pharmacological means is sufficient to increase the potency of AMPH for uptake inhibition in a single brain region (Calipari *et al.* 2013), and here we extend those findings to show that regional variations in DAT function within the same animal correspond to different AMPH potencies, measured both at the DAT and as AMPH effects on exocytotic release.

While previous work has studied the effects of acute AMPH dosing across striatal regions *in vivo,* here we examined the effects of AMPH directly on dopamine terminals. Many *in vivo* studies have administered AMPH doses between 1 and 10 mg/kg (Ramsson *et al.* 2011a,b), which has been shown to result in relatively low brain AMPH levels (Siciliano *et al.* 2014). Although a 10 mg/kg i.p. dose is considered a high dose because it produces robust behavioral activation, the drug only reaches peak brain levels of ~200 nM and is completely cleared within 100 min (Daberkow *et al.* 2013; Siciliano *et al.* 2014). Previous work determined that at these doses or concentrations, AMPH does not cause reverse transport (Daberkow *et al.* 2013; Siciliano *et al.* 2014). Thus, the effects of AMPH at these low concentrations are likely more relevant to therapeutic use of the drug, where AMPH does not induce dopamine efflux and concomitant vesicular dopamine depletion. Conversely, translational models of human AMPH abuse (i.e., self-administration) typically use intravenous (instead of i.p.) dosing that is repeated over the course of many hours. This can lead to an accumulation of AMPH and much higher AMPH brain levels that are sustained for the duration of the self-administration session, which is likely better modeled by the concentrations studied in the current manuscript.

Although previous literature has compared AMPH potency across striatal regions *in vivo* via microdialysis (Hernandez *et al.* 1987; Sharp *et al.* 1987; Di Chiara and Imperato 1988; Pehek *et al.* 1990; Robinson and Camp 1990; Kuczenski *et al.* 1991; Kuczenski and Segal 1992) and voltammetry (Ramsson *et al.* 2011a,b), here we aimed to dissect the mechanism of the differential effects of AMPH across regions by examining AMPH directly on dopamine terminals. While *in vivo* studies are particularly relevant for determining drug effects in an intact brain, modulation of the dopamine system by multiple afferents and interneuron populations makes it difficult to attribute differences across regions to a specific mechanism. The present data provide a potential mechanism for greater AMPH-induced dopamine overflow (Hernandez *et al.* 1987; Pehek *et al.* 1990; Robinson and Camp 1990; Kuczenski *et al.* 1991; Kuczenski and Segal 1992; but see Sharp *et al.* 1987; Di Chiara and Imperato 1988) and AMPH-induced increases in basal dopamine levels (Ramsson *et al.* 2011a,b) in dorsal versus ventral regions of the striatum. Although there are other regional differences which may influence AMPH potency, DAT function is the most tenable mechanism for disparities

in AMPH potency across regions. This hypothesis is supported by findings showing increased AMPH potency in animals with elevated DAT levels (Calipari *et al.* 2013). There are several possible, though not opposing, explanations of the relationship between DAT levels and AMPH potency. Firstly, it is likely that elevations in DAT levels facilitate the ability of AMPH to enter the terminal. This could lead to increased cytoplasmic AMPH which is likely to increase vesicular access and allow for augmented movement of dopamine into the cytoplasm. Higher DAT levels may also increase reverse transport, a process which has been shown previously to be DAT dependent (Jones *et al.* 1998; Fleckenstein *et al.* 2007; Sulzer 2011). Together, it is likely that these effects allow increased AMPH effects on both reverse transport and vesicular depletion with increasing DAT levels.

Gradual increases in DAT levels and dopamine uptake rates across the ventral–dorsal axis of the striatum have been observed in multiple species, including rodents, non-human primates, and humans (Coulter *et al.* 1996; Yeghiayan *et al.* 1997; South and Huang 2008; Calipari *et al.* 2012), although the underlying cause of the gradient is not entirely clear. The most parsimonious explanation is that dorsal regions receive greater dopamine innervation and thereby a higher density of terminals, and thus transporters, per cubic millimeter of tissue than the ventral regions. This is supported by the similar gradient of dopamine tissue content (Cragg *et al.* 2000) and electrically evoked release (Cragg *et al.* 2000; present findings) across striatal subregions. Another explanation may be the relative contribution of terminals from dopamine neurons of the substantia nigra or ventral tegmental area, since the former consistently exhibit higher uptake rates and DAT expression than the latter (Shimada *et al.* 1992; Blanchard *et al.* 1994), and there is a gradual shift from predominantly substantia nigra innervation in dorsal CPu to predominantly ventral tegmental area innervation in the NAc shell (Beckstead *et al.* 1979). In addition, differences in the local microenvironment surrounding dopamine terminals could contribute to variations in DAT expression, trafficking, and function. For example, activation of D2 autoreceptors on presynaptic terminals by high extracellular dopamine levels increases the maximal uptake rate (V_{max}) for dopamine (Meiergerd *et al.* 1993; Batchelor and Schenk 1998). There are many types of receptors on dopamine terminals (e.g., nicotinic acetylcholine, GABA-B, serotonin 1B receptors, etc.), and differences in the number and activity of these receptors may alter uptake rates as well, either directly through intracellular signaling or indirectly through alterations in dopamine release.

One important implication of the current findings is that differential AMPH potency across striatal subregions may drive habitual drug taking during AMPH self-administration. It is thought that operant behavior is controlled by two processes which differentially contribute to the addiction process (Everitt and Robbins 2013). The first is goal-directed responding, which is maintained by the direct association between the response and the reinforcer. After extensive drug experience, a second type of responding occurs, which is referred to as habitual responding. Habitual responding results from repeated pairings of the reinforcer with the cues that signal its availability and eventually the cues alone can maintain responding. The dorsal CPu has been shown to mediate habitual behaviors as well as cue–reward associations that drive responding on higher order schedules of reinforcement with complex or sequential cues (Ito *et al.* 2002; Gabriele and See 2011; Everitt and Robbins 2013). In contrast, the NAc is involved in goal-directed responding, such as early acquisition of self-administration behavior and consummatory drug-taking in rodents, as well as the euphoric/rewarding 'high' experienced in human psychostimulant users (Drevets *et al.* 2001; Everitt and Robbins 2013). Thus, higher AMPH potency in dorsal regions may result in increased habitual, as compared to goal-directed, behaviors during AMPH abuse. This hypothesis is supported by findings demonstrating that cocaine, which elevates dopamine more robustly in the NAc than the CPu (Segal and Kuczenski 1992), engenders more goal-directed and less habitual behavior as compared to AMPH (Richardson and Roberts 1996; Carroll and Lac 1997; Crombag *et al.* 2008). Indeed, AMPH has been shown to induce greater responding on higher order self-administration schedules compared to cocaine (Richardson and Roberts 1996; Crombag *et al.* 2008) despite cocaine engendering higher consummatory responding in rodents (Carroll and Lac 1997) and greater self-reported positive subjective effects in humans (Fischman *et al.* 1976; Comer *et al.* 2013).

Here we showed that AMPH potency differs across striatal dopamine terminal regions with the dorsal CPu being the most impacted and the NAc shell the least impacted by AMPH. Additionally, we demonstrated that differential AMPH potency across regions is directly correlated with dopamine uptake rates, providing a possible mechanism for these effects. These findings also provide a potential mechanism for the increased development of habitual behaviors during AMPH self-administration as compared to cocaine and may have important clinical implications. Individuals suffering from attention deficit hyperactivity disorder or post-traumatic stress disorder, which are often comorbid with addiction, have been shown to have elevated DAT levels in the striatum (Madras *et al.* 2005; Hoexter *et al.* 2012). Thus, understanding the relationship between DAT levels and AMPH potency could: (i) allow the identification of populations at higher risk for addiction and provide an avenue for preventative treatment, and (ii) allow for more personalized treatment with the DAT as a pharmacotherapeutic target. Further, regional differences in AMPH potency may explain the differential behavioral repercussions of cocaine and AMPH exposure, and understanding the underlying factors responsible for the

consequences of specific stimulants may allow for the development of more targeted therapeutics.

Acknowledgments and conflict of interest disclosure

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All experiments were conducted in compliance with the ARRIVE guidelines.

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