Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Amphetamine Mechanisms and Actions at the Dopamine Terminal Revisited

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Review of Daberkow et al.

Amphetamine (AMPH) exerts its rewarding and reinforcing effects by elevating extracellular dopamine (DA) and prolonging DA receptor signaling in the striatum. Traditionally, AMPH has been characterized as a DA releaser that elevates DA by three major mechanisms. First, it is a substrate for the DA transporter (DAT) that competitively inhibits DA uptake; second, it facilitates the movement of DA out of vesicles and into the cytoplasm; and third, it promotes DAT-mediated reverse-transport of DA into the synaptic cleft independently of action-potential-induced vesicular release (Fleckenstein et al., 2007). *In vitro* studies on the mechanisms of AMPH action have demonstrated that AMPH causes DA release, which can result in saturation of DA receptors (Richfield et al., 1989), and eventually lead to depletion of intracellular DA stores (Jones et al., 1998; Schmitz et al., 2001). In their recent publication in *The Journal of Neuroscience*, Daberkow et al. (2013) propose a new model of AMPH action that not only extends accepted mechanisms, but also calls some traditional hypotheses of AMPH action into question. Their main conclusion is that low-dose (1 mg/kg) AMPH administration facilitates both electrical- and cue-evoked vesicular DA release and does not change DA-dependent behaviors *in vivo*, contrary to what one would expect if AMPH were depleting terminals as shown *in vitro*.

Daberkow et al. (2013) used fast-scan cyclic voltammetry in freely moving rats to determine the effects of AMPH on DA system kinetics and signaling. First, they electrically stimulated ascending dopamine fibers to elicit DA release in the striatum. Instead of reducing stimulated DA release as would be expected if DA in nerve terminals was depleted, intaperitoneal (i.p.) injection of AMPH (1 and 10 mg/kg) enhanced DA release compared with a predrug baseline (Daberkow et al., 2013, Fig. 1). They completed their recordings after DA release returned to the predrug baseline level, at 2 h postinjection (Daberkow et al., 2013, Fig. 2*A*). *In vitro* studies suggest that this time frame is sufficient to result in at least partial depletion of terminals, particularly at higher concentrations (Jones et al., 1998); however, instead of observing reduced release, Daberkow et al. (2013) observed increased release. It is important to note that because DA release had not stabilized at this time, it is possible that a longer recording time would have revealed depletion of DA from terminals, and a concomitant decrease in stimulated DA release. In addition to increasing electrically evoked release, AMPH increased both the frequency and amplitude of spontaneous DA release events, even at the highest dose of AMPH, further supporting the idea that AMPH increases DA release. Additionally, their analysis of stimulated DA release following AMPH administration suggested that increased amplitude did not result from DAT reversal (Daberkow et al., 2013, Fig. 3). If AMPH was depleting terminals via reverse transport, one would predict a decrease in the amplitude of vesicular DA release independent of frequency (Daberkow et al., 2013, Fig. 4). Together, these data suggest that AMPH does not deplete DA terminals as suggested by *in vitro* studies.

Daberkow et al. (2013) extend this work in behaving animals to test whether AMPH can facilitate DA release in response to cues that predict rewarding stimuli in the environment. To test this, the authors used a discriminative stimulus task whereby a distinct audiovisual cue indicated the availability of a lever, that when pressed, resulted in the delivery of a sugar pellet reward. Once animals were trained to associate the appropriate audiovisual cue with the reward-paired lever, DA was monitored in a drug-naive state for all animals. Subsequently, DA responses were monitored following saline, low dose AMPH (1 mg/kg), or high dose AMPH (5 mg/kg) injections. In the drug naive trials, phasic DA release was time-locked to the cue that indicated the presentation of the reward-paired lever. This cue-induced DA release is the signal

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that encodes the motivational value of rewards and thus is essential for goal-directed behaviors, such as correctly responding to obtain a reward following the presentation of a reward-paired lever. If AMPH depletes terminals and causes saturation of postsynaptic DA receptors, cue-evoked DA release and corresponding goal-directed behavior should be disrupted. There was no significant effect on the magnitude of cue-induced DA release or goal-directed behavior in the group receiving saline injections. The low dose of AMPH (1 mg/kg) enhanced the absolute magnitude of cue-induced DA signals without affecting behavior. Conversely, the high dose (5 mg/kg) disrupted DA signals by causing DA release events that were not time-locked to the cue and thus effectively abolished goal-directed behavior (Daberkow et al., 2013, Fig. 6).

Based on these results, Daberkow et al. (2013) argue that differences between *in vivo* and *in vitro* findings are attributable to differences in the preparation such as stimulation parameters, and AMPH acting on different vesicular storage pools. However, another possibility is that the concentration of AMPH at the terminal is different between cited *in vitro* studies and the present study. The authors argue that in *in vitro* studies AMPH ubiquitously depletes terminals, but they cite work using concentrations of AMPH that exceed what could be reached with a single i.p. injection (Honecker and Coper, 1975). The brain concentration of AMPH in the present study can be estimated by determining the amount of uptake inhibition caused by AMPH *in vivo* and comparing it to *in vitro* studies. The authors report a 200 and 400% increase in the DA uptake parameter apparent K_m, reflecting a decreased affinity of DA caused by competitive inhibition following a single injection of 1 mg/kg and 10 mg/kg AMPH, respectively. Increases of this magnitude *in vitro* correspond to ~100–300 nM AMPH. This is particularly important because at such concentrations there is no depletion of DA terminals *in vitro*. Ferris et al. (2012) showed that AMPH does not reduce DA release in brain slices until concentrations reach 1–3 μM, which are much higher than is likely reached in the present study even at the higher injected dose, confirming some of the Daberkow et al. (2013) results. Therefore, the differences in AMPH effects probably do not result from the preparation *per se,* but rather from differences in the concentration of AMPH interacting with the DA terminal.

While Daberkow et al. (2013) make a good case for considering a new mechanism of AMPH based on vesicular release, it is important not to disregard the mechanism of DAT-mediated reverse transport. Repeated AMPH administration, which is often used as a model of AMPH abuse, was not discussed. Self-administration studies in which animals administer AMPH over an extended period result in much higher levels of drug intake (Di Ciano et al., 2002). Indeed, animals with a history of AMPH self-administration show reduced DA following a period of abstinence, suggesting that at these high, abuse-relevant doses, AMPH is depleting terminals of DA *in vivo* (Di Ciano et al., 2002). This emphasizes the point that AMPH leads to depletion of DA at synaptic terminals both *in vitro* and *in vivo*, but only at high doses.

It is possible that the AMPH-induced augmentation of stimulated DA release, as seen in the Daberkow et al. (2013) study, occurs at low doses because the reverse-transport effects of AMPH are not engaged. AMPH-induced reverse-transport of DA via the DAT relies on sufficient cytoplasmic concentrations of DA. AMPH-induced depletion of vesicles has been suggested to result from its properties as a weak base that increases the pH in vesicles, thus leading to the release of DA from vesicles into the cytoplasm (Sulzer et al., 1992). Once in the cytoplasm, DA can be released into terminals via AMPH-induced reversal of the DAT. It is possible that at low doses, AMPH cannot reach sufficient concentration within vesicles to alter pH to the extent necessary for efflux. The inability of AMPH to produce efflux from vesicles at low does would cause pharmacological effects resembling those of traditional DAT blockers. Indeed, Daberkow et al. (2013) state that the AMPH-induced elevation of DA seen in their study is similar to what has been demonstrated previously for cocaine, a prototypical DAT blocker, which facilitates vesicular release.

The paper by Daberkow et al. (2013) also speaks to the importance of the ratio of cue-induced phasic DA release relative to the pre-cue DA baseline in the facilitation of goal-directed behaviors. The authors report that AMPH (1 mg/kg) increased the absolute peak height of the DA signal (mean ± SEM), which included both the cue-induced phasic signal and the pre-cue DA baseline (Daberkow et al., 2013, Fig. 7). Close inspection of the figure suggests that the increased peak magnitude of DA in the AMPH group relative to saline or pre-drug controls largely results from a greater pre-cue DA baseline (Daberkow et al., 2013, Fig. 7C, baseline epoch), rather than greater cue-induced phasic release *per se* (Daberkow et al., 2013, Fig. 7C, cue epoch). Indeed, when examining the ratio of phasic release to the pre-cue baseline, the increase in the signal between groups appears to be virtually identical. If, as suggested in the manuscript, the total magnitude of DA increase was relevant for behavior, one would expect improved behavioral performance in the discriminative stimulus task, as DA elevations are responsible for the execution of goal-directed behaviors. However, in the behavioral task, animals that received AMPH (1 mg/kg) did not perform differently than control animals. This suggests that phasic/baseline ratios, rather than absolute DA magnitude, drive shifts in goal-directed behavior. This is supported by the high dose (5 mg/kg) condition, in which the phasic/baseline ratio and goal-directed behavior were disrupted concurrently (Daberkow et al., 2013, Fig. 7*D*). It is likely that the ability to process phasic events depends on the contrast between the phasic signal and the baseline DA level that was present before presentation of the cue.

In conclusion, we believe the study by Daberkow et al. (2013) has two important implications that were not discussed by the authors. First, the facilitation or disruption of goal directed behaviors is dependent on the magnitude of phasic DA release relative to baseline DA levels (i.e., phasic/baseline), rather than general increases in overall DA levels (i.e., phasic + baseline). Second, they show that AMPH dose is critical in determining its acute effects *in vivo* and *in vitro*, because AMPH may act similar to a blocker at low concentrations and as a releaser at high concentrations. This latter point is particularly relevant when considering the low-dose therapeutic use of AMPH compared with higher doses used in AMPH abuse. Given the possible shift in mechanism with increasing AMPH concentrations, it is important to amend, but not to disregard, the traditional models of AMPH action.

References

Daberkow DP, Brown HD, Bunner KD, Kraniotis SA, Doellman MA, Ragozzino ME, Garris PA, Roitman MF (2013) Amphetamine paradoxically augments exocytotic dopamine release and phasic dopamine signals. J Neurosci 33: 452–463. CrossRef Medline

Di Ciano P, Blaha CD, Phillips AG (2002) Inhibition of dopamine efflux in the rat nucleus ac-

cumbens during abstinence after free access to d-amphetamine. Behav Brain Res 128: 1–12. CrossRef Medline

Ferris MJ, Calipari ES, Mateo Y, Melchior JR, Roberts DC, Jones SR (2012) Cocaine self-administration produces pharmacodynamic tolerance: differential effects on the potency of dopamine transporter blockers, releasers, and methylphenidate. Neuropsychopharmacology 37:1708–1716. CrossRef Medline

Fleckenstein AE, Volz TJ, Riddle EL, Gibb JW, Hanson GR (2007) New insights into the mechanism of action of amphetamines. Annu Rev Pharmacol Toxicol 47:681–698. CrossRef Medline

Honecker H, Coper H (1975) Kinetics and metabolism of amphetamine in the brain of rats of different ages. Naunyn Schmiedebergs Arch Pharmacol 291:111–121. CrossRef Medline

Jones SR, Gainetdinov RR, Wightman RM, Caron MG (1998) Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. J Neurosci 18:1979–1986. Medline

Richfield EK, Penney JB, Young AB (1989) Anatomical and affinity state comparisons between dopamine D1 and D2 receptors in the rat central nervous system. Neuroscience 30: 767–777. CrossRef Medline

Schmitz Y, Lee CJ, Schmauss C, Gonon F, Sulzer D (2001) Amphetamine distorts stimulation-dependent dopamine overflow: Effects on D2 autoreceptors, transporters, and synaptic vesicle stores. J Neurosci 21:5916–5924. Medline

Sulzer D, Pothos E, Sung HM, Maidment NT, Hoebel BG, Rayport S (1992) Weak base model of amphetamine action. Ann N Y Acad Sci 654:525–528. CrossRef Medline