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Distinctions Between Dopamine Transporter Antagonists Could Be Just Around the Bend

L. Keith Henry[1,3‡] and Randy D. Blakely[1,2,4]

Departments of [1]Pharmacology and [2]Psychiatry

Centers for [3]Structural Biology and [4]Molecular Neuroscience

Vanderbilt University

Nashville, TN 37232-8548

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Running Title: Dopamine Transporter Antagonists

‡To Whom Correspondence should be addressed:

Current Address:

L. Keith Henry, Ph.D.

Dept of Pharmacology

Vanderbilt University School of Medicine

Nashville, TN 37232-8548

Tel: 615-322-0122

FAX:615-936-3040

Address after April 2008:

Dept of Pharm, Physiol and Therap

University of North Dakota School of

Medicine and Health Sciences

Grand Forks, ND 58203

Tel: 701-777-6221

Fax: 701-777-4490

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Abstract

Abuse of psychostimulants such as cocaine and amphetamines has a tremendous social and economic impact. While replacement therapies are offered for addiction to opioids, nicotine and alcohol, there is no approved replacement treatments for psychostimulant addiction. Recent studies on an emerging group of benztropine- and remcazole-based compounds provide hope that replacement therapies for cocaine and amphetamine addiction may come in the near future. A new study now investigates the molecular interaction of the benztropine and remcazole compounds with their target, the dopamine transporter and provides an intriguing explanation as to why use of these compounds, unlike cocaine, do not lead to locomotor stimulation and drug discrimination behaviors in animal models.

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Drug use typically begins as a voluntary decision that with repeated exposure, is superseded by involuntary craving and pursuit of illicit agents despite adverse physical and social outcomes(Volkow and Li, 2005). Overall, the social and economic impact of drug addiction is enormous. Estimates indicate the presence of nearly 30 million drug addicts in the US and Western Europe alone(Pouletty, 2002). Cocaine and amphetamine addicts face distinct challenges in kicking their habits as, unlike those seeking treatment for alcoholism or opiate addiction, no clinically proven substitution therapies are available for psychostimulant abuse. The psychostimulant properties of cocaine are considered to be mediated primarily through the blockade of the dopamine (DA) transporter (DAT) that results in increased levels of extracellular DA. This concept has gained perhaps its strongest support recently from the work of Chen and coworkers(Chen et al., 2006) using transgenic mice bearing engineered mutations in DAT that reduce cocaine potency *in vitro* where cocaine becomes a motor depressant and loses the ability to support conditioned place preference behavior (CPP). One often discussed, but so far impractical, concept for reducing the grip of cocaine on the dopamine transporter (DAT) in human addicts is the use of a non-reinforcing blocker of cocaine binding that does not itself perturb DA transport(Lin and Madras, 2006). This ambitious goal first requires the identification of molecules that can bind to DAT in a mode distinct from that of cocaine and most other DAT antagonists. In a new study (see pg X), Loland et al. find that benztropine- and rimcazole-based compounds, recently reported as potential medications for cocaine addiction(Dutta et al., 2003), are found to promote non-cocaine-like conformations of DAT and could be a pivotal step toward realizing psychostimulant substitution therapy.

We have long known that cocaine and other psychostimulants can potentiate DA signaling by binding to DATs and preventing DA clearance from the synapse . The study by Loland et al supports a more nuanced view that the physiological result of DAT blockade depends heavily upon the temporal

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profile of the drug's access to the transporter and on the conformational changes produced after binding. This new work stems from a recent report of benztropine (BZT) and remcazole-based DAT inhibitors that yield behavioral outcomes in animal studies quite distinct from tests with cocaine(Rothman et al., 2008). These differences include lack of locomotor stimulation and an inability to discriminate the drug from saline. Given that blockade of DAT by cocaine is thought to be the primary mechanism whereby locomotor stimulation and psychostimulant discrimination are thought to arise, the absence of these behaviors in mice treated with these analogs, despite exhibiting high-affinity for DAT, seems paradoxical. Loland et al., tackle this issue with molecular approaches that can define conformational states in DAT pre- and post antagonist binding.

The adoption of distinct conformational changes in DAT upon antagonist binding compared to DA-bound DAT has already been suggested using protease sensitivity assays(Gaffaney and Vaughan, 2004). Now, Loland et al. probe conformational changes in DAT, both before and after binding of the BZTs, by monitoring accessibility of an introduced cysteine at residue I159 to cysteine-reactive methanethiosulfonates (MTS). Reaction of DAT I159C with MTS reagents has been used by others to infer changes in protein conformation, such as one might envision with the rotation of a helix or the shift from open to closed status of an extracellular gate(Chen and Rudnick, 2000; Loland et al., 2004). Accessibility and modification of I159C by MTS reagents results in loss of DA transport which the authors believe reflects a state where an outer gate limiting access to the DA permeation pathway is open; inability of MTS reagents to react with I159C and inactivate transport reports that this gate is closed. Results from the Loland et al MTS studies suggests that several BZT analogs bind to a conformation of DAT with the outer gate closed. This is in direct contrast with cocaine, which appears to bind to, and stabilize, the "open" state of the transporter.

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But is there any evidence for structural parallels to these "gates"? Prior to the pioneering studies of Yamashita on the high-resolution structure of a bacterial homolog of DAT, $LeuT_{Aa}$(Yamashita et al., 2005), this was just speculation. In the $LeuT_{Aa}$ structure, charged residues have been identified that can serve as either extra- or intra-cellular gates (Figure 1). The breaking and stabilization of salt-bridges between these charged residues is postulated to promote either the entry or exit of substrate (e.g. DA) from the transporter. So the prediction is that whereas a DAT/cocaine complex binds to a conformation with the outer pair of residues separated ("gate open"), binding of some BZT-based compounds promotes a DAT conformation where the salt-bridge is stabilized ("gate closed"). Of course these gates are expected to open and close across the course of the transport cycle, though structures that validate this concept have yet to be published. Additionally, though candidate residues for the DAT gates are easy to identify, proof of their use in such a mechanism awaits a high resolution structure of DAT in the absence and presence of antagonists.

Loland and colleagues provide additional evidence that cocaine and the BZT compounds bind different forms of DAT using a clever pharmacological strategy that takes advantage of the unique properties of a previously studied Y335A mutant(Loland et al., 2004; Loland et al., 2002). The Y335A mutant has been shown to promote a change in conformation of DAT, consistent with a shift of the protein to an "inward facing" conformation. Using competition binding analyses, Loland et al demonstrate that several BZT compounds exhibit an apparent increase in binding affinity to this "inward facing" state whereas cocaine's affinity is actually decreased. Although there might be other explanations for these effects, the data strongly suggest that BZT-based compounds bind a form of DAT conformationally distinct from that bound by cocaine. Mason and colleagues recently described that novel norepinephrine (NE) transporter (NET) antagonists, typified by desvenlafaxine sulfate, exhibit sensitivity to membrane disruption that classical NET antagonists such as desipramine and nisoxetine do

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not(Mason et al., 2007). Perhaps the latter observations can also be explained by distinct conformations stabilized by different classes of NET antagonists, only some of which are allowed after membrane disruption.

This differential binding between BZTs and cocaine at DAT is interesting, but perhaps of greater significance is the link discovered by the authors between binding characteristics of the compound and the compound's impact on animal behavior. When mice or rats are used to assess locomotor behavior or drug discrimination, the propensity of a BZT analog to favor the Y335A-induced "inward facing" DAT conformation appears predictive of a concomitant loss of cocaine-like behaviors. The unique characteristics of these compounds assure their further study as prospective leads for psychostimulant substitution therapy. The potential generality of the Loland et al findings to those interested in other transporters such as the antidepressant-sensitive NET or serotonin transporter (SERT) are easy to see and may, ultimately, be of larger impact given the high lifetime incidence of mood disorders commonly treated with NET or SERT blockers. Besides the opportunities highlighted by Loland et al for DAT targeted therapies, perhaps novel antidepressants that act faster, have better efficacy, and that permit safer discontinuation are also just around the bend.

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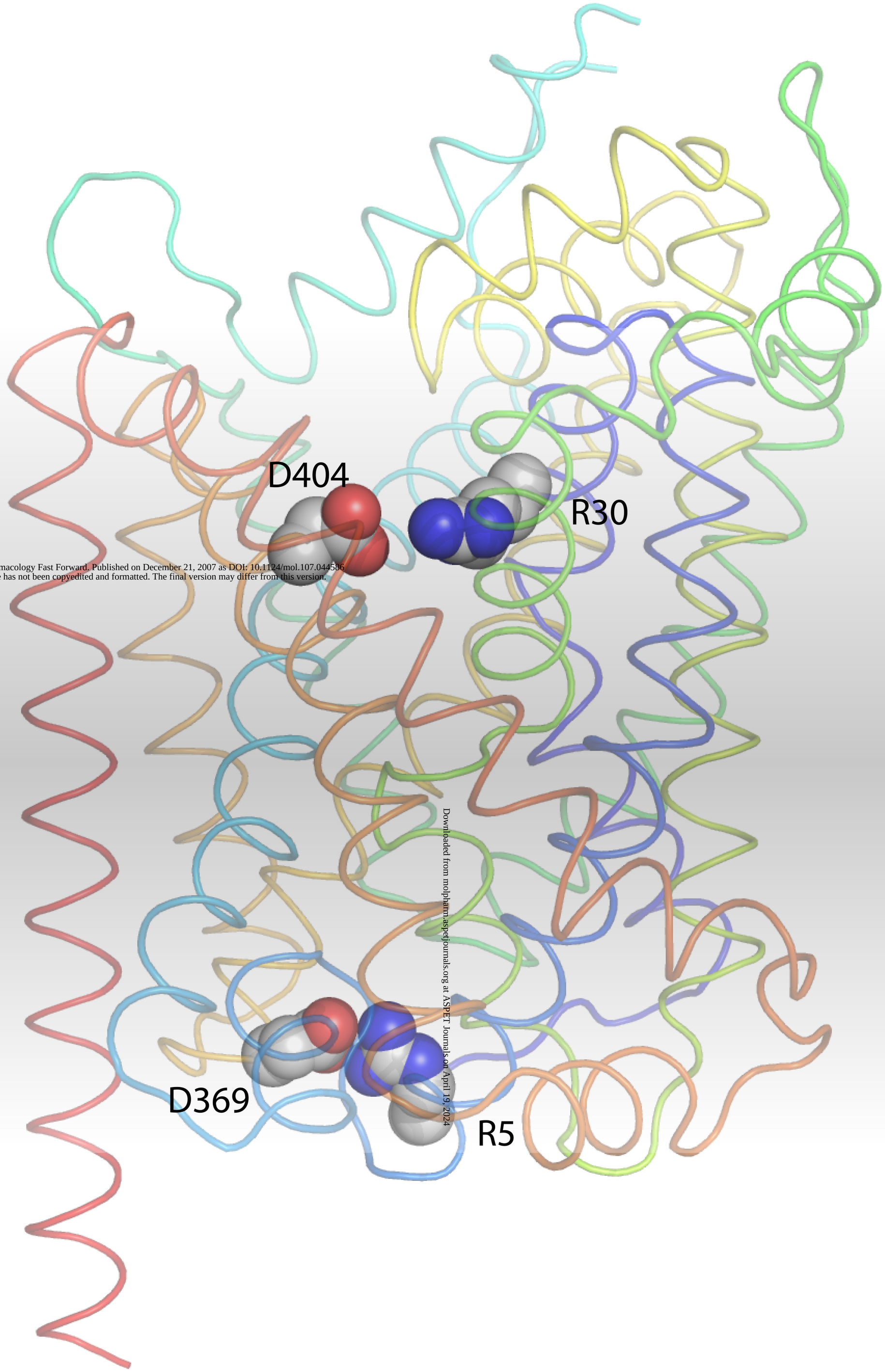
Figure Legend

Figure 1. Illustration of the inner and outer gates in LeuT structure. LeuT structure is displayed in cartoon mode with the side chains of residues R30, D404 and R5, D369 representing the inner and outer gates, respectively, shown in space filling mode. Figure was generated using LeuT coordinates (2A65) in Pymol (DeLano Scientific LLC, San Carlos, CA)



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