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# The promiscuity of the dopamine transporter: Implications for the kinetic analysis of [$^3$H]serotonin uptake in rat hippocampal and striatal synaptosomes

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**Abstract**

Evidence indicates that monoaminergic neurotransmitter transporters are promiscuous, transporting substrates other than their cognate neurotransmitters. For example, serotonin is transported by the dopamine transporter (DAT) under conditions in which serotonin transporter (SERT) activity is eliminated (e.g., pharmacological inhibition). We performed a kinetic analysis of [$^3$H]serotonin uptake in rat striatal synaptosomes (expressing DAT and SERT) and hippocampal synaptosomes (expressing SERT, but not DAT). Nonspecific [$^3$H]serotonin uptake was defined as the amount of uptake remaining in the presence of fluoxetine (10 μM) or paroxetine (0.05 μM). In hippocampal synaptosomes, $K_m$ and $V_{max}$ values for [$^3$H]serotonin uptake did not differ whether fluoxetine or paroxetine was used to define nonspecific uptake. However, in striatal synaptosomes, both $K_m$ and $V_{max}$ values for [$^3$H]serotonin uptake were greater when fluoxetine, rather than paroxetine, was used to define nonspecific uptake. These data suggest that, at the concentrations employed, fluoxetine inhibits serotonin uptake at both DAT and SERT, whereas paroxetine only inhibits serotonin uptake at SERT. Thus, when DAT is inhibited by GBR 12909, kinetic parameters for serotonin uptake via SERT in striatum are not different from those obtained in hippocampus. These findings have important implications regarding the analysis of monoaminergic reuptake in brain regions exhibiting heterogeneous transporter expression.


*Keywords:* Neurotransmitter transporter; Reuptake; Kinetics; Dopamine; Serotonin

## 1. Introduction

An accumulating body of literature has demonstrated that monoaminergic neurotransmission occurs through both classical one-to-one synaptic mechanisms and extra-synaptic, volume transmission (for review see Zoli et al., 1999). In addition, immunocytochemical evidence reveals that monoamine transporters are located peri-synaptically on nerve terminals and along axonal membranes (Pickel et al., 1996; Hersch et al., 1997; Zhou et al., 1998; Tao-Cheng and Zhou, 1999); both the nature of volume transmission and the extra-synaptic localization of monoamine transporters underlies the transport of monoamines between neuronal populations through multiple transporter types. For example, serotonin (5-HT) is readily transported, under certain conditions, into dopamine (DA) neurons in brain regions expressing transporters for both the dopamine transporter (DAT) and serotonin transporter (SERT) (Stamford et al., 1990; Jackson and Wightman, 1995; Zhou et al., 2002, 2005; Callaghan et al., 2005). Recently, exogenous

*Abbreviations:* 5-HT, serotonin; ANOVA, analysis of variance; DA, dopamine; DAT, dopamine transporter; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NET, norepinephrine transporter; SERT, serotonin transporter.

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5-HT was shown to be cleared from striatum at a faster rate than from hippocampus following a pressure injection of equivalent signal amplitude, as indicated by electrochemical detection, into both brain regions (Callaghan et al., 2005). Results of the latter study demonstrated that the increased striatal 5-HT clearance rate was due to significant 5-HT uptake through DAT as this effect was blocked by application of a DAT inhibitor, GBR 12909. In another study, Zhou et al. (2005) showed that striatal dopaminergic terminals will take up 5-HT and subsequently release both 5-HT and DA when SERT is inhibited by a serotonin-specific reuptake inhibitor (SSRI) and extracelluar levels of 5-HT are elevated. In addition, 5-HT was found to be taken up, stored, and subsequently released by catecholaminergic neurons in rabbit olfactory tubercle under conditions in which serotonin transporter (SERT) activity was inhibited by the SSRI, citalopram (Suarez-Roca and Cubeddu, 2002). Riddle et al. (2003) extended these findings by showing that ceramide, an agent known to alter the phosphorylation state of transporter proteins, increases 5-HT uptake and reduces DA uptake through DAT. 5-HT immunoreactivity has been demonstrated in DA neurons in the midbrain of both SERT knockout mice and wild-type mice treated with the SSRI, paroxetine (Zhou et al., 2002). The promiscuity of transporter proteins may have significant clinical implications considering the breadth of psychoactive drugs in which the antagonism of monoaminergic transporter activity serves as a primary mechanism of action (e.g., antidepressants, drugs of abuse).

In pre-clinical pharmacological studies, the promiscuity of transporter proteins for substrate is especially relevant when analyzing and comparing the kinetic parameters of monoamine reuptake across brain regions. In rat striatum, for example, SERT- and DAT-expressing afferent inputs emerge from the midbrain dorsal raphe nuclei and substantia nigra, respectively (Graybiel and Ragsdale, 1983). Although there is dense serotonergic innervation to rat hippocampus originating in the raphe nuclei, this region is essentially devoid of dopaminergic innervation (Fuxe et al., 1985; Donnan et al., 1989; Mennicken et al., 1992). Although numerous studies have investigated the kinetics of 5-HT reuptake under varying environmental and pharmacological conditions (O'Reilly and Reith, 1988; Asano et al., 1997; Kokoshka et al., 1998; Wells et al., 1999; Martin et al., 2000; Pollier et al., 2000; Nandi et al., 2004; Nightingale et al., 2005) and in varying rat strains (Martin et al., 2000; Fernandez et al., 2001, 2003), few studies have considered the impact of the expression of both DAT and SERT in brain regions such as striatum and the potential contribution of DAT to the analysis of 5-HT uptake kinetics. The heterogeneous expression of DAT and SERT in specific brain regions is also an important factor with regard to selecting specific transporter inhibitors and concentrations to define nonspecific uptake. The use of a highly selective inhibitor at a concentration sufficient to saturate the transporter of interest, without inhibiting other monoaminergic transporters, is desirable, yet can be difficult to obtain given the lack of inhibitors demonstrating absolute selectivity.

In the present study, the kinetic parameters ($K_m$ and $V_{max}$) of 5-HT uptake were determined in rat hippocampal and striatal synaptosomal preparations. To evaluate the contribution of DAT to [$^3$H]5-HT uptake in striatum, synaptosomes were incubated in the absence or presence of the selective DAT inhibitor GBR 12909 (Rothman et al., 1989). Nonspecific [$^3$H]5-HT uptake in both hippocampal and striatal synaptosomes was defined as the amount of uptake remaining in the presence of either fluoxetine or paroxetine.

## 2. Materials and methods

### 2.1. Animals

Male Sprague–Dawley rats (225–250 g, Harlan Laboratories, Indianapolis, IN) were housed two per cage with food and water available ad libitum. Rats were maintained under temperature- and humidity-controlled conditions on a 12-h/12-h light/dark cycle in the Division of Laboratory Animal Resources in the College of Pharmacy at the University of Kentucky. All experimental procedures were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and approved by the Institutional Animal Care and Use Committee at the University of Kentucky. All efforts were made to minimize animal suffering and to reduce the number of animals used. There were no available alternatives to using rat brain preparations for these in vitro analyses.

### 2.2. Drugs and chemicals

[$^3$H]5-HT [5-[1,2-$^3$H(N)-Hydroxytryptamine creatinine sulfate] (specific activity, 27.1 Ci/mmol) was purchased from PerkinElmer Life Sciences (Boston, MA).

5-Hydroxytryptamine creatinine sulfate (5-HT), 1-(2-*bis*(4-fluorphenyl)-methoxy)-ethyl-4-(3-phenyl-propyl) piperazine (GBR 12909) HCl, fluoxetine HCl, pargyline HCl, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), bovine serum albumin, catechol, and L-ascorbic acid were purchased from Sigma–Aldrich (St. Louis, MO). D-Glucose was purchased from Aldrich Chemical Co. (Milwaukee, WI). Paroxetine HCl was generously provided by Beecham Pharmaceuticals (Surrey, UK).

### 2.3. Synaptosomal preparation and [$^3$H]5-HT uptake

Synaptosomes were prepared from hippocampus or striatum obtained from different rats. For the kinetic analysis of [$^3$H]5-HT uptake into hippocampal synaptosomes, in which there is no DAT expression (Swanson et al., 1987), two sets of experiments were performed in which either: (1) fluoxetine or (2) paroxetine was used to define nonspecific [$^3$H]5-HT uptake. For the kinetic analysis of [$^3$H]5-HT uptake into striatal synaptosomes, in which there is significant DAT expression, four sets of experiments were performed in which synaptosomes were incubated with: (1) fluoxetine to define nonspecific [$^3$H]5-HT uptake and GBR 12909 to inhibit DAT function, (2) fluoxetine to define nonspecific uptake without DAT inhibition, (3) paroxetine to define nonspecific uptake and GBR 12909 to inhibit DAT, or (4) paroxetine to define nonspecific uptake without DAT inhibition. A concentration of 10 μM fluoxetine was selected based on its common use in kinetics studies of 5-HT uptake inhibition (Asano et al., 1997; Martin et al., 2000; Pollier et al., 2000; Zhang et al., 2002; Fernandez et al., 2003). In an effort to achieve selective 5-HT uptake at SERT, a concentration of 0.05 μM paroxetine was selected, which is 200-fold higher than its $K_i$ at SERT (0.1–0.4 nM; for review see Nemeroff and Owens, 2003) and about 20-fold lower than its $K_i$ at DAT (1.0 μM; Nemeroff and Owens, 2003; Owens et al., 2001; Koch et al., 2002). Kinetic analyses inform mechanistic evaluations of pharmacotherapeutic candidates as well as current pharmacotherapies. As indicated above, the concentrations employed in these in vitro mechanistic analyses, particularly with respect to those chosen to define nonspecific uptake, are based on in vitro studies and do not have direct clinical relevance to therapeutic concentrations determined in clinical studies. For example, 10 μM fluoxetine is an order of magnitude greater than the estimated brain free drug concentration of fluoxetine, and 0.05 μM paroxetine is close to the minimum estimated brain free drug concentration (Zhou et al., 2007).

Rats were killed by rapid decapitation, brains were removed, and hippocampi or striata were quickly dissected on ice. Hippocampi or striata were