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Research Report

Effect of environmental enrichment on dopamine and serotonin transporters and glutamate neurotransmission in medial prefrontal and orbitofrontal cortex

Mahesh Darna[a], *Joshua S. Beckmann*[b], *Cassandra D. Gipson*[b,c], *Michael T. Bardo*[b], *Linda P. Dwoskin*[a,*]

[a]*Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536, USA*
[b]*Department of Psychology, University of Kentucky, Lexington, KY 40536, USA*
[c]*Department of Neurosciences, Medical University of South Carolina, Charleston, SC 29425, USA*

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ABSTRACT

Recent studies have reported that rats raised in an enriched condition (EC) have decreased dopamine transporter (DAT) function and expression in medial prefrontal cortex (mPFC), as well as increased d-amphetamine-induced glutamate release in nucleus accumbens compared to rats raised in an isolated condition (IC). In these previous studies, DAT function and expression were evaluated using mPFC pooled from four rats for each condition to obtain kinetic parameters due to sparse DAT expression in mPFC. In contrast, accumbal glutamate release was determined using individual rats. The current study extends the previous work and reports on the optimization of DAT and serotonin transporter (SERT) functional assays, as well as cell surface expression assays using both mPFC and orbitofrontal cortex (OFC) from individual EC or IC rats. In addition, the effect of d-amphetamine on glutamate release in mPFC and OFC of EC and IC rats was determined using in vivo microdialysis. Results show that environmental enrichment decreased maximal transport velocity (V_{max}) for [^{3}H]dopamine uptake in mPFC, but increased V_{max} for [^{3}H]dopamine uptake in OFC. Corresponding changes in DAT cell surface expression were not found. In contrast, V_{max} for [^{3}H]serotonin uptake and cellular localization of SERT in mPFC and OFC were not different between EC and IC rats. Further, acute d-amphetamine (2 mg/kg, s.c.) increased extracellular glutamate concentrations in mPFC of EC rats only and in OFC of IC rats only. Overall, these results suggest that enrichment produces long-lasting alterations in mPFC and OFC DAT function via a trafficking-independent mechanism, as

Abbreviations: DAT, dopamine transporter; SERT, serotonin transporter; EC, enriched condition; IC, isolated condition; mPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; V_{max}, maximal transport velocity

*Correspondence to: Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 789 S. Limestone Street, 465 Biological Pharmaceutical Complex, Lexington, KY 40536-0596, USA. Fax: +1 859 257 7585.
E-mail address: ldwoskin@email.uky.edu (L.P. Dwoskin).

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well as differential glutamate release in mPFC and OFC. Rearing-induced modulation of DAT function and glutamate release in prefrontal cortical subregions may contribute to the known protective effects of enrichment on drug abuse vulnerability.

1. Introduction

Previous research reveals that both genetic and environmental factors contribute to individual differences in drug abuse vulnerability (Bardo et al., 2013). Preclinical models have been developed to evaluate pre-existing individual differences and to understand the neurobehavioral mechanisms that underlie drug abuse vulnerability. Enrichment and isolation housing conditions have been used to evaluate the role of environmental factors in drug abuse vulnerability. Rats reared in an enriched condition (EC) with social cohorts and novel objects exhibit decreased locomotor activity in an inescapable novel environment compared to those raised in an isolated condition (IC; Green et al., 2003; Zhu et al., 2004). EC rats exhibit decreased intravenous self-administration of low unit doses of d-amphetamine across repeated sessions compared to IC rats (Bardo et al., 2001). Also, environmental enrichment decreases impulsivity, which has been linked to drug abuse (Perry et al., 2005, 2008; Wood et al., 2006; Baler and Volkow, 2006). Taken together, environmental enrichment may protect against vulnerability to drug abuse.

The enrichment-induced reduction in vulnerability to abuse d-amphetamine and other drugs may be mediated, at least in part, by altered dopamine (DA), serotonin (5-HT) and glutamate function in brain regions implicated in reward (Zhu et al., 2004; Brenes et al., 2008; Rahman and Bardo, 2008; Bardo et al., 2013). EC rats exhibit an increased density of DA-immunoreactive fibers innervating striatum compared to IC rats (Wallace et al., 1992). In nucleus accumbens, EC rats have greater glucose utilization, as well as greater amphetamine-stimulated DA and glutamate release compared to IC rats (Bardo et al., 1999; Gonzalez-Lima et al., 1994; Rahman and Bardo, 2008). This suggests that enrichment may sensitize the mesolimbic reward system, perhaps leading to a compensatory decrease in the amount of drug that is self-administered by EC rats compared to IC rats.

Extracellular DA and 5-HT concentrations are regulated primarily by the DA transporter (DAT) and serotonin transporter (SERT), respectively. DAT and SERT are major targets for addictive drugs, which alter the function and trafficking of these transporters (Zahniser and Sorkin, 2009; Ramamoorthy et al., 2011). Environmental enrichment during development alters DAT function in a brain-region specific manner (Zhu et al., 2004). In mPFC, V_{max} for [^{3}H]DA uptake is decreased in EC rats compared to IC rats, with no differences between EC and IC rats in nucleus accumbens or striatum. Decreased DAT function in mPFC in EC rats is associated with a reduction in cell surface expression in EC compared with IC rats (Zhu et al., 2005). In these previous studies, DAT function and expression were evaluated using mPFC pooled from four rats to obtain kinetic parameters due to the sparse DAT expression in mPFC. Enrichment-induced alterations in DAT function have not been examined in other subregions of prefrontal cortex such as orbitofrontal cortex (OFC). OFC has been implicated in mediating impulsivity (Perry et al., 2011), which plays a role in drug abuse vulnerability. Moreover, EC rats exhibit less impulsivity relative to IC rats (Perry et al., 2008). Thus, assessment of enrichment-induced alterations in DAT function in OFC is important for elucidating the neural mechanisms underlying both the reduced impulsivity and reduced drug abuse vulnerability in EC compared to IC rats.

Recent evidence also indicates that the serotonergic system in mPFC and OFC is involved in impulsivity (Winstanley et al., 2010). Whether alterations in these cortical systems play a role in the protective effect of environmental enrichment on drug abuse vulnerability is not known. Impulsivity determined in the delay discounting task has been associated with changes in extracellular 5-HT concentrations in mPFC and extracellular dihydroxyphenylacetic acid (DOPAC) in OFC (Winstanley et al., 2006). However, relatively little is known about the effect of environmental enrichment on SERT function and cellular localization. In one study, the selective SERT inhibitor citalopram was ineffective in reversing aggressive behavior in isolated mice compared to group housed mice (Rilke et al., 2001). However, it remains to be determined if enrichment alters basal SERT function in either mPFC or OFC.

Although extracellular DA regulates the activity of dopaminoceptive glutamate neurons (Svenningsson et al., 2004), relatively little is known about enrichment-induced changes in glutamate systems. Compared to EC rats, IC rats have decreased glutamatergic tone mediated by mGluR2 receptors in mPFC (Melendez et al., 2004), suggesting a role of glutamatergic signaling in environment-induced effects. In addition, pretreatment with MK-801, a noncompetitive NMDA receptor antagonist, prevents amphetamine-induced increases in extracellular glutamate concentrations in nucleus accumbens of EC rats relative to IC rats (Rahman and Bardo, 2008). Although environment-induced alterations in glutamatergic activity in prefrontal cortex may play a role in enrichment-induced alterations in impulsivity (Melendez et al., 2004), the role of glutamate release and its regulation in response to psychostimulants, such as amphetamine, in the prefrontal cortex of EC and IC rats has not been investigated.

The current study investigated the effect of environmental enrichment on DAT and SERT function and expression in mPFC and OFC as potential mechanisms underlying the previously observed enrichment-induced protective effects on drug abuse vulnerability. In addition, basal and amphetamine-induced extracellular glutamate concentrations in mPFC and OFC were determined using in vivo microdialysis to determine whether glutamate transmission in these prefrontal regions is associated with the enrichment-induced protective effects.