

Short communication

The ratio of striatal D1 to muscarinic receptors changes in aging rats housed in an enriched environment

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Accepted 16 May 2000

Abstract

The enriched environment (EC) causes morphological plasticity in striatal cells that express D1 and D2 receptors. We used radioligand binding assays to examine whether EC produces plasticity in striatal receptor density and receptor density ratios. After 30 days of EC, 2-year-old rats had a higher ratio of D1 to muscarinic receptors in striatum relative to singly housed rats. Assays also showed trends for a greater ratio of D1 to cannabinoid receptors and a greater density of D1 receptors in striatum after EC. D2 receptor density was unaffected by the EC condition. © 2000 Elsevier Science B.V. All rights reserved.

Theme: Neural basis of behavior

Topic: Aging

Keywords: Plasticity; Dopamine; D2 receptor; Physical activity

Dopamine synthesis [14] and dopamine receptors (D1 [11]; D2 [14,26,27]; both D1 and D2 [20,30]) are reduced with age in striatum. In rats, the decline cannot be accounted for solely by cell loss, and therefore reflects in part a reduction of receptors on surviving neurons [16]. The same is true for human dopamine transporters [2] whose decline correlates with a decline in D2 receptors [32]. Although both decline with age, the correlation between the two is independent of age. This suggests that both dopaminergic molecular targets may be regulated by a mechanism that is independent of age.

The mechanism regulating the reduction in dopamine receptors is not well-understood. Loss of dopaminergic input in addition to pharmacological manipulation can influence the number of dopamine receptors in the striatum [1,4,25,33]. Here we questioned whether functional plasticity, such as age-related slowing of behavior, might

contribute to the down-regulation of these molecular targets in elderly animals. Conversely, we also questioned whether active complex behaviors could up-regulate these dopaminergic targets. In support of this latter hypothesis, the medium spiny neurons in the dorsolateral striatum, which express dopaminergic receptors, undergo changes in spine density and an increase in multiple-head spines in response to rearing in an enriched environment (EC; [5,6]). The present experiment was designed to test the hypothesis that exposure to an enriched environment and related changes in behavior can influence the striatum of aged rats. We were particularly interested in whether or not this manipulation could increase dopaminergic receptors.

A number of studies have indicated an interactive relationship between other transmitter systems and the effects of dopaminergic receptor activation in striatum. Of particular interest are muscarinic receptors, which co-localize with DA receptors on striatonigral GABA neurons [22] and modulate DA receptor-mediated effects [8,12,17,28,29]. That Parkinson's disease can be treated by either dopaminergic agonists or cholinergic antagonists

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suggests an importance for the balance between cholinergic and dopaminergic systems [22]. Cannabinoid receptors are also co-localized with dopamine receptors in striatal neurons [19] and the two receptor systems have a functional interaction [7,13]. These functional interactions lead us to also question whether the ratio of DA (D1 and D2) to muscarinic and cannabinoid receptors would change after exposure to an enriched environment.

To test whether or not the EC condition had effects on any of these receptor systems or the proportional relationships of these receptor types, we housed aged rats in an EC environment for 1 month, then performed binding assays for D1, D2, cannabinoid (CB1), and muscarinic receptors on striatal homogenates.

Male Fisher 344 rats (2 years old) were housed together in large toy-filled cages (EC; $n=10$) or alone in standard tub cages (IC; $n=10$). Food was scattered throughout the EC cage and available from two cups attached to the side of the cage. The placement of the food cups and water bottles was changed daily to encourage the EC rats to explore. During the last hour of the dark cycle of each day, the rats were transferred from their EC cage to a similar cage with different toys for 1 h so that toys in their home cage could be rearranged. Rats in the IC group were handled daily for 5 min to control for the handling of the EC rats during cage transfers. During the treatment condition, four animals from each group died due to age. After 30 days in the treatment condition, rats were decapitated, the brains were removed and immediately frozen in isopentane maintained at -30°C with dry ice. Frozen brains were coated with OCT and frozen again in isopentane. Tissue was stored at -70°C until preparation of homogenates.

After thawing at room temperature, brain regions were immediately dissected and homogenized using a Polytron. Binding assays were conducted using 1 mg (wet weight) of original tissue in a total volume of 0.25 ml of Krebs saline buffered with 50 mM Tris, pH 7.4. D1 receptors were determined in striatum using [^3H]SCH 23390 with SCH39166 (1 μM) to define non-specific binding. D2 receptors were determined in striatum using ^3H -labeled *N*-methylspiperone with haloperidol (1 μM). Muscarinic receptors were determined using [^3H]quinuclidinyl benzilate with bntropine (1 μM). Cannabinoid CB1 receptors were determined using [^3H]SR141716A with WIN 55,212-2. The range of concentrations of [^3H]SR141716A was approximately 0.1–20 nM. The other radioligands were

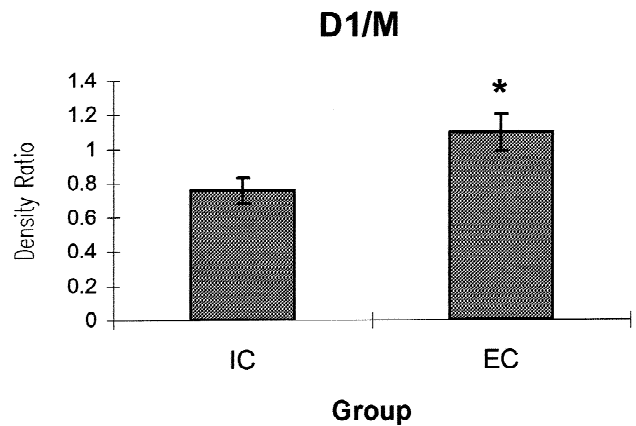


Fig. 1. D1 to muscarinic receptor density ratio. The ratio of striatal D1 to muscarinic receptor density was significantly greater in the EC rats relative to the IC rats. $*P<0.05$.

used between approximately 0.3 and 60 nM. Incubations were conducted for 90 min at 30°C on a shaking water bath, and were terminated by filtration on a Brandel cell harvester. Filters were counted with a liquid scintillation counter at about 40% efficiency.

A one-way ANOVA revealed a trend for the EC group to have 27% more D1 receptors in striatal homogenates than the IC group ($F(1,10)=3.37$, $P<0.10$, see Table 1). The ratio of D1 to muscarinic receptors in EC rats was 43% higher than in the IC rats, a significant difference ($F(1,10)=6.56$, $p<0.05$, see Fig. 1). A trend indicated that the EC group had an increased ratio of D1 to cannabinoid receptors relative to the IC group (see Table 1, $F(1,10)=4.28$, $p=0.06$). The enriched environment had no apparent effects on D2 receptors or on the ratio of D2 receptors to muscarinic or cannabinoid receptors (see Table 1).

In this experiment, housing in an enriched environment influenced the ratio of D1 to muscarinic receptor density in striatum. The EC group also had a trend toward a higher ratio of D1 to cannabinoid receptors. The data indicate a change in the relative expression of receptors that modulate dopaminergic cell activity. These results are consistent with earlier studies showing that behavioral treatment conditions can influence the level of dopamine receptors in adult [10,23] and aging rats [24]. The present results are similar to findings that the EC increased D1 receptors but not D2 receptors in the occipital cortex of young rats [21]. Taken together these studies suggest that the EC condition is capable of influencing D1 receptors across the lifespan.

Table 1
D1 and D2 receptor density and receptor density ratios^a

	D1 Density	D1/cannabinoid density ratio	D2 density	D2/muscarinic density ratio	D2/cannabinoid density ratio
IC	44.00 (4.82)	0.729 (0.09)	66.33 (6.41)	1.16 (0.13)	1.20 (0.23)
EC	56.17 (4.56)*	1.11 (0.26)**	60.83 (10.22)	1.26 (0.27)	1.22 (0.26)

^a Striatal receptor density (pmol/g tissue, wet weight) and density ratios for the inactive (IC) and enriched condition (EC). Mean (S.E.M.). $*P=0.10$; $**P=0.06$.

In the present study, there was no indication that EC influenced D2 receptor density, or the ratio of D2 receptor density to other receptor types. Previous studies using treadmill training to influence DA receptor density reported an increase in D2 receptor density [10,23] and a dampening of age-related reductions in D2 receptors [24], but have not addressed changes in D1 receptors. In light of the previous findings, the present data suggest that plasticity in D2 receptor density requires a greater duration and intensity of physical activity than that available in the EC condition. If the change in the D1/muscarinic receptor ratio in the present study is the result of increased physical activity in the EC, then perhaps D1 receptors are influenced by subtler and/or shorter periods of physical activity than D2 receptors. Alternatively, the increase in D1 to muscarinic receptor ratio could be related to the novelty and natural reinforcers available in the EC condition. Dopaminergic systems in the nucleus accumbens, included in the striatal homogenates, have been implicated in novelty-seeking behavior [9,31] and in learning new 'likes' and 'dislikes' [3].

Although a number of studies report age-related reductions in D1 receptors in striatum [14,20,30], several studies have failed to find such reductions [18,26]. If D1 receptors decrease with age, then the EC condition in the present study may have reversed an age-related decline. Alternatively, if D1 receptors are maintained over the lifespan, then the EC condition may have increased the D1/muscarinic receptor ratio above baseline. It is also possible that a small proportion of the difference between the EC and IC condition is related to a slight decline in D1/muscarinic receptor ratio caused by the isolation in the IC condition. We assume that rats in any aging studies of DA receptors were housed in standard conditions that are equivalent to the social housing condition. Previous experiments using the EC, IC and social housing condition have shown that a small proportion of the EC and IC differences in other variables is related to a reduction of those variables by isolation [15].

The change in the ratio of D1/muscarinic receptor density in striatum is of interest because a balance of muscarinic to DA receptors has previously been suggested to be important for proper movement [22]. Future studies will be required to assess functional consequences of the enriched environment that may be related to the change in the ratio of D1/muscarinic receptors. The trend for a change in the ratio of striatal cannabinoid to DA receptors indicates a subtle change in the balance of these interacting receptor systems as well.

Acknowledgements

This work was supported by Department of Energy/OBER and MH57845. We would like to thank Beatrice Pyatt for help with receptor assays, and Jack Travis for

helping care for the animals. The Dept. of Laboratory Animal Resources generously supplied the enriched environment cages.

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