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Dopamine receptor alterations with aging in mouse and rat corpus striatum

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Several recent experimental studies have demonstrated age-related changes in various neurotransmitter systems of mammalian brain [1–7]. Although the synaptic mechanisms of both cholinergic and dopaminergic systems are apparently altered in old animals, dopaminergic synapses seem particularly vulnerable in aging. Samorajski [1] reported decreased striatal dopamine synthesis in old rats. Jonec and Finch [8] measured reduced dopamine uptake in hypothalamic and striatal synaptosomes from aged mice. Govoni *et al.* [9] and Puri and Volicer [10] have shown that dopamine-stimulated adenyl cyclase activity is significantly lower in the striata of 20- to 24-month-old rats than it is in 2- to 3-month-old animals. Similar decreases were found in several brain regions, including striatum, of aged rabbits [11]. Furthermore, Bertler [12] and Carlsson and Winblad [13] reported a significant decline in the dopamine content of human striatum at senesence. To describe such changes in greater detail, we examined the age-related alterations of dopaminergic neurotransmission directly at the receptor level by examining the binding properties of radiolabeled haloperidol, a potent dopamine receptor antagonist and neuroleptic agent. Binding was assayed in homogenates of corpora striata dissected from mouse and rat brains from various age groups encompassing young adult to senescent animals [14].

Specific binding of [^{3}H]haloperidol (New England Nuclear Corp., Boston, MA, sp. act 19.74 Ci/mmole) to post-synaptic dopaminergic receptors was determined by the method of Burt *et al.* [15] in homogenates of isolated caudate-putamen (striatum) of rat (Charles River, CD) and mouse (C57BL/6J) brains. The tissues were coded such that the experimenter had no prior knowledge of the age group. Homogenates were prepared by a 1:20 (w/w) dilution of 0.32 M sucrose buffered with 15 mM Tris–HCl, pH 7.4. Total binding of [^{3}H]haloperidol and non-specific binding in the presence of 1 μM excess unlabeled haloperidol were determined in 15 mM Tris–HCl buffer, pH 7.4, containing 5 mM NaEDTA and 1 mM ascorbate. Following a 30-min incubation of tissue (0.2 to 0.4 mg protein) and ligand at 22°, filtration through Whatman GF/B glass fiber filters and rapid washing with 12 ml of buffer separated free and bound ligand. Radioactivity on the filters was determined in a Packard Tri-Carb scintillation counter at an efficiency of 38 per cent. Receptor number and affinity were determined by Scatchard analysis of specific binding of five concentrations (0.4 to 9.8 nM) of [^{3}H]haloperidol. Protein concentrations were measured by the method of Lowry *et al.* [16] using bovine serum albumin (BSA) as a standard.

In the first group of mouse brains, specific binding of [^{3}H]haloperidol was assessed at 2.8 nM, a saturating ligand concentration, in striata of animals 4, 10, 24 and 32 months of age. Four or five animals were independently studied in each age group. Preliminary experiments demonstrated that the K_d for [^{3}H]haloperidol was 1.35 nM in normal mouse brain striatal homogenates. The animals were decapitated, and the brains were rapidly removed, placed on ice, and dissected as outlined by Glowinski and Iverson [17]. As indicated in Table 1, the striatal tissues were kept frozen at −20° or −80° for 2–6 weeks prior to the binding studies. Table 1 shows that specific [^{3}H]haloperidol binding increased 8-fold in the mature (24-month) mice compared

Table 1. [^{3}H]Haloperidol binding to homogenates of mouse striatum: Effect of age on receptor number at a saturating ligand concentration (2.8 nM)*

Age (months)	[^{3}H]Haloperidol binding† (fmoles/mg protein)
4 (5)‡	31.1 ± 26.6
7 (4)§	139.0 ± 5.0
10 (5)	142.8 ± 17.2
24 (5)	268.0 ± 26
32 (4)	175.3 ± 55
32 (4)§	187.0 ± 6.0

* Each value is the mean ± S.E.M.
† ANOV: F = 13.06* (degrees of freedom = 16). Student's non-paired t-test: 4 months vs 24 months, t = 4.7, P < 0.01; 24 months vs 32 months, t = 2.4, P < 0.05.
‡ Number of brains per age group.
§ These striata were stored −80° for 6 weeks, whereas all others were stored at −20° for 2 weeks before use.

to the young (4-month) animals. A significant decrease in binding was measured in the senescent, 32-month-old mouse brains relative to the 24-month-old animals. The very small amounts of tissue available from these brains (about 20 mg of striatum) precluded a Scatchard analysis. In statistical evaluation of these data, the analysis of variance revealed an overall significant effect of aging on dopamine receptors (F = 13.06*), and subsequent Student's t-test for non-paired data revealed a significant elevation of binding from 4 to 24 months (t = 4.7, P < 0.01) and a significant decrease thereafter (t = 2.4, P < 0.05).

A second group of animals consisted of four rats each at two ages, 6 and 26 months. The brain tissues were handled as above with the dissected striata stored at −80° for about 2 weeks before use. The larger amount of striatal tissue available from this species (about 60 mg wet weight) permitted Scatchard analysis on each brain to determine receptor number (B_{max}) and receptor affinity (K_d). No significant or consistent change could be measured in the size (tissue wet weight) of striata from rats of the different age groups studied, and the tissues were diluted to maintain as constant a protein/wet weight ratio as possible. Table 2 shows that aging increased the number of specific [^{3}H]haloperidol binding sites 3-fold, while the apparent receptor affinity decreased 7-fold. Scatchard analysis of binding in this range of drug concentrations yielded a single binding component.

The results of the present studies indicate that alterations in both the number and affinity of dopaminergic receptors in cerebral striatal regions occur naturally during aging. This is probably not a species-specific phenomenon, as the elevation of binding sites was seen in both rat and mouse. The changes are not due to a generalized change in brain protein content, since [^{3}H]haloperidol binding was similarly related to age whether calculated on the basis of protein or wet weight of tissue, especially as the tissue homogenates were prepared in a constant volume/tissue weight ratio. Unfortunately, a complete longitudinal study is lacking for the rat, as animals in other age groups were not available for this study. Direct comparison of the two species is also limited by the lack of a Scatchard analysis of the binding data in mouse brain.

Transmission at dopaminergic synapses in the senescent CNS is most likely compromised by both pre- and postsynaptic alterations at the morphologic, physiologic, and neurochemical levels. Our present studies confirm and extend those of Govoni *et al.* [9, 18] that receptor affinity for dopaminergic ligands is reduced in aged, 30-month-old rats. Thus, a diminished receptor sensitivity apparently accompanies aging. In contrast with our results, however, those authors did not observe variations in receptor number in rat striatum, although receptor number was reduced by half in pituitary from the aged animals. One possible explanation for this difference may be the diphasic nature of the relationship of dopamine receptor number to age, as evidenced from the longitudinal study presented in Table 1. Because Govoni *et al.* [9, 18] pooled animals into two wide age ranges, significant receptor alterations may have become cancelled. In the present study, age-related increments occurred from 3 to 10 months of age and also from 10 to 24 months of age, but declined thereafter. It is possible that loss of dopaminergic afferents during the incremental phase may provoke receptor compensation by elevation of binding capacity [19] (denervation supersensitivity) from maturity (10 months) to older age (24 months). It has been suggested, however, that the ability to develop an adrenergic supersensitivity response is diminished in aged rats [4, 20].

Recently, Severson and Finch [21] found a decrease in the B_{max} with no change in the K_d for another dopaminergic antagonist, [^{3}H]spiroperidol, in the striata of 28-month-old rats. Misra *et al.* [22] also reported a decrease in [^{3}H]spiroperidol binding in the striata of 25-month-old compared with 5-month-old rats, with no change in receptor affinity. These data contrast both with the results of the experiments reported in Tables 1 and 2 and with the data presented by Govoni *et al.* [9, 18]. It must be concluded that this diversity of variations in dopamine receptors observed in rats and mice of different age groups remains to be defined by a detailed, uniform longitudinal study on a large number of animals raised under constant environmental factors. Numerous influences on the experimental data, including diet, environmental stress and method of killing, to name but a few, have not yet been studied.

The decrease in receptor number at 32 months of age in the present study may represent their disappearance from, or the loss of, striatal neurons on which they reside [23]. This receptor decrement, coupled with the loss of receptor affinity, may produce significant communication deficits at dopaminergic synapses in aged animals. Recently, Marshall and Berrios [24] observed that movement disorders of aged rats, like those seen in young adult animals with injured brain dopaminergic neurons, are markedly reversed by administration of apomorphine or L-dopa. These authors suggested that age-related alterations in brain dopaminergic systems may be responsible for some of the motor disturbances associated with senescence. Though considerably more evidence is required for such a correlation, the results of the present study are compatible with that hypothesis.

In summary, direct binding studies with the dopamine receptor antagonist [^{3}H]haloperidol revealed alterations in both the number and affinity of dopaminergic receptors in

Table 2. [^{3}H]Haloperidol binding to homogenates of rat striatum: Effect of senescence on receptor number and affinity determined by Scatchard analysis

Age (months)	K_d (nM)	B_{max} (fmoles/mg protein)
6 (4)*	1.8 ± 0.7	796 ± 123
26 (4)	11.9 ± 4.8	2261 ± 325
t^+ =	2.074	4.211
P =	0.05	0.0031

* Number of brains per age group.
† Student's non-paired t-test.

striatal regions from aged mouse and rat brains. In the mouse, receptor number increased between 4 and 24 months and then decreased sharply at 32 months of age. Rat striata exhibited a 3-fold increase in the number of haloperidol binding sites and a 7-fold decrease in affinity. A more detailed study is needed to correlate receptor deficits with the motor and mental dysfunctions of senescence.

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