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Effect of Duration of Haloperidol Treatment on DA Receptor Supersensitization in Aging C57BL/6J Mice

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Apomorphine-induced behavior, striatal [^{3}H]spiperone binding, and striatal choline acetyltransferase (ChAT) activity were assessed in 6½, 13, and 27–30 month-old male C57BL/6J mice following 0, 30, 60 or 90 days treatment with the dopaminergic (DA) antagonist haloperidol. Both apomorphine-induced behavior and [^{3}H]spiperone binding (B_{max}) increased linearly with duration of haloperidol treatment, with no detectable age difference in the degree of supersensitization, although basal receptor density declined with age. Middle- and old-aged mice showed prolonged stereotypic behavior relative to young mice, suggesting slower apomorphine clearance. No differences in ChAT activity were detected with either age or duration of haloperidol treatment. Although the group means of binding and behavior were highly related, the within group correlations were poor. Overall, the results suggest that aged animals are capable of DA receptor supersensitization when given a sufficient stimulus — in this case, relatively long treatment regimes. Previously reported deficits in neuroleptic-induced supersensitization in old mice may be confined to relatively short treatment periods at low doses.

INTRODUCTION

Age-related alterations in dopaminergic regulation may be important determinants of the increased incidence of major motoric side-effects of neuroleptic drugs in the elderly[3,13,35]. In addition, such changes may contribute to the onset of symptomotology of some age-related motoric diseases, particularly Parkinson's disease[18,19]. One phenomenon occurring in response to chronic dopamine antagonist treatment with neuroleptic drugs is dopaminergic receptor supersensitivity. Binding sites for dopamine (DA)-selective ligands increase after such treatment[7,46,58]. Electrophysiological[64,75] and behavioral sensitivity to DA agonists is enhanced[70]. Age-related alterations in compensatory supersensitivity could potentially explain the increased risk of both drug-induced Parkinson's and tardive dyskinesia.

Dopamine receptor density in the basal ganglia is known to decrease with age[61,62,71], but age-related changes in DA receptor supersensitization are much less clear. We previously reported that a 21-day haloperidol treatment failed to produce DA supersensitization in 24-month-old C57BL/6J mice. Supersensitization was evaluated both by behavioral response to the DA agonist, apomorphine, and by specific binding of [^{3}H]spiperone[53]. Joseph et al., however, reported that aging rats show no deficit in supersensitization produced by electrolytic[33] or 6-hydroxydopamine-induced lesion[34] of the nigro-striatal pathway. The same investigators observed a 40–50% increase in [^{3}H]spiperone binding which was similar across ages[34]. A number of other reports have been mixed, some showing a modest deficit in supersensitization in the aging rodent[29,44,45] and others showing no deficit or even increases in supersensitization[54].

It is difficult to resolve the differences in these studies. A wide range of experimental protocols have been employed, e.g. species, strain, method of induction of supersensitization. One likely possibility is

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that aging rodents retain the capability for receptor proliferation, but require a more severe treatment to initiate the supersensitization response. In order to address this question, we examined apomorphine-induced stereotypic behavior and striatal [^{3}H]spiperone binding in 3 ages of mice following 0, 30, 60 or 90 days treatment with haloperidol. In addition, since striatal cholinergic mechanisms are tightly coupled to DA, and may participate in compensation for chronic DA blockade[26,27,41,49,66,69] we examined striatal choline acetyltransferase activity of these same mice.

MATERIALS AND METHODS

Animals and design

C57BL/6J mice obtained from Jackson Laboratories (Bar Harbor, ME) served as subjects for this experiment. Young animals were obtained at 2½ months of age and the middle-aged and old animals were obtained at 8–12 months as retired breeders. Until the time of the experiment animals were housed in a limited access colony with a 12:12 h day–night cycle. Ages for the 3 groups were 6½, 13 and 27–30 months at the end of the experiment. Within age-groups, mice were assigned randomly to vehicle (30 day), 30-, 60- or 90-day haloperidol treatment groups. Starting times for the 3 haloperidol treatment durations were staggered so that all groups were tested and sacrificed over a 4-day period. Mice were withdrawn from haloperidol for a period of 7 days before behavioral testing occurred. On the day after behavioral testing, mice were sacrificed by cervical dislocation and the striata rapidly dissected and frozen on dry ice for later analysis of [^{3}H]spiperone binding and choline acetyltransferase activity.

Chronic haloperidol treatment

Haloperidol (gift of McNeil Pharmaceutical, Spring House, PA) was administered in the drinking water to avoid the stress of daily injections which are not tolerated well by the older mice[53]. Concentration of the drug in the drinking water at the start of the experiment was 20 μg/ml, and over the course of the experiment was adjusted by group for monitored water intake, to provide an approximate daily dose of 2.5 mg/kg. Typically, water intake decreased somewhat over the first 1–3 days and then stabilized, requiring little adjustment thereafter. Haloperidol was dissolved in a small amount of 1% lactic acid and then mixed thoroughly with the drinking water. Vehicle animals received only the lactic acid.

Apomorphine-induced behavior

The behavioral response to apomorphine was assessed in 15 × 15 × 15 cm enclosures of wire mesh with solid plexiglass tops and floors. After a 10-min habituation period, 1.0 mg/kg apomorphine hydrochloride (Merck, Sharpe and Dohme, Rahway, NJ) was administered intraperitoneally in a cold (0.9%) saline vehicle. Apomorphine was prepared immediately before each run and kept on ice during the short period in which the animals were weighed. Thirty-second observations were made every 6 min following injection of apomorphine, for a period of 1 h. At each observation a rating of the drug-induced behavior was made according to a 7-point rating scale developed on the C57BL/6J mouse[52,53]. This scale gives a log-linear dose response curve over a wide range of doses. Twelve mice were tested simultaneously in 1 of 4 sessions per day: 2 between 09.00 and 13.00 h and 2 between 14.00 and 17.00 h. All groups were equally represented in each run so that time of day and run variables were balanced across groups.

All behavioral data were analyzed by analysis of variance with a split plot design (Age × Haloperidol Duration × Time After Apomorphine Injection) within a random variable for testing session (blocks)[37]. The method of unweighted means was employed when necessary to accommodate unequal n. Criterion for statistical significance was 0.05.

[^{3}H]spiperone binding

Individual mouse striata (about 20 mg) were homogenized using a Polytron (setting 6, 10 s) in 5 ml ice-cold 20 nM 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (HEPES, pH 7.5) containing 154 mM NaCl (HEPES–isosaline). Homogenates were centrifuged 10 min at 20,000 *g* and the supernatant saved for determination of choline acetyltransferase (ChAT) activity. The pellet was rehomogenized, as above, in 3 ml ice-cold HEPES-isosaline containing 5 mM ethylene diamine tetraacetic acid (EDTA) and incubated 15 min at 37 °C. Crude membranes were again pelleted by centrifugation and the pellet homogenized in 3 ml ice-cold HEPES–isosa-

line and used immediately in the binding assay. All age and treatment groups were included in each assay run. In addition, age groups were rotated in analysis order on a run-to-run basis to balance potential order of analysis effects. No such effects were observed. D-Butaclamol (Ayerst Research Laboratories, Montreal) and [^{3}H]spiperone (26.7 Ci/mmol, New England Nuclear, Boston, MA) were diluted in HEPES–isosaline containing ascorbic acid (Sigma, St. Louis, MO) and bovine serum albumin (Sigma) at the final assay concentrations of 0.26 mM and 2.0 μg/ml, respectively.

The radiochemical purity of [^{3}H]spiperone (97%) was periodically checked by high-performance liquid chromatography (HPLC) using a Vydac C-18 column (4.6 × 25 cm) and a solvent system of trifluoroacetic acid and acetonitrile.

Binding assays were conducted in duplicate in glass 12 × 75 mm culture tubes. Six concentrations of [^{3}H]spiperone were diluted to give final assay concentrations of 30–1000 pM in a 50 μl addition. D-Butacolamol for non-specific binding was dissolved in a small amount of acetic acid before dilution in buffer to give a final assay concentration of 2 μM in a 100-μl aliquot. The concentration of acetic acid during the binding reaction was < 1 nM. Tubes for total binding received 100 μl of buffer only. The binding reaction was initiated by the addition of striatal homogenate (about 40 μg protein in 100 μl) and mixing. The binding reaction (10 min, 37 °C) was terminated by the addition of 5 ml ice-cold 10 mM Tris-HCl (pH 7.5) containing 154 mM NaCl and rapid filtration through Schleicher and Schuell No. 30 glass fiber filters. Binding tubes and filters were washed with an additional 5 ml of wash buffer. ^{3}H trapped on the filters was determined by liquid scintillation spectometry at 40.2% efficiency. Homogenate protein content was estimated using bovine serum albumin as the standard[5].

Saturation data were linearized by Scatchard transformation[56] and the B_{max} and K_d obtained from the least squares linear regression fit. Statistical analysis was similar to that of the behavioral data. In order to maintain a balanced design, binding values for 3 lost samples were estimated by Yates iterative procedure[37]. Total and error degrees of freedom were accordingly reduced by 3.

Choline acetyltransferase assay

The enzyme source used was the S2 fraction from the membrane preparation described above. This supernatant was aliquoted and frozen at –20 °C immediately following centrifugation. Individual samples were then thawed for each series of assays. ChAT is stable under these conditions for months, and the variation between different series of assays was usually less than 5%.

The assay protocol is modified from that of Fonnum[23]. Briefly, the assay mixture contained (final conc.): 100 μM [^{3}H]acetyl-CoA, 300 mM NaCl, 10 mM choline chloride, 1 mM EDTA and 0.1 mM eserine sulfate in 25 mM sodium phosphate buffer (pH 7.4). The assays were started by adding the enzyme to the preincubated substrate mixture. All assays were carried out at 37 °C for 12 min. Final volume was 100 μl.

The reaction was stopped by the addition of 1.25 ml of ice-cold acetonitrile containing 5 g/liter sodium tetraphenyl borate. A 0.5-ml aliquot of this solution was then added to an equal volume of H_2O in a scintillation vial, followed by the addition of 5 ml of a toluene-based scintillation cocktail containing 0.05% 2,5-diphenyloxazole (PPO) and 0.02% 1,4 bis[2-(5-phenyloxazolyl)]benzene (POPOP). After gentle mixing, the vials were counted in a Beckman LS 7500 liquid scintillation counter, with an efficiency of 25%.

RESULTS

The results in general, were clearly consistent with the hypothesis that given a sufficiently severe treatment, old mice show equal dopaminergic supersensitization as do young mice.

Apomorphine-induced behavior

Mean stereotype rating as a function of time after apomorphine for the different ages and haloperidol treatment groups are shown in Fig. 1. Stereotype ratings increased with haloperidol duration ($F = 6.25$; df = 2,73; $P < 0.005$) and no differential effect of haloperidol duration as a function of age was detected (Age × Haloperidol Duration; $F < 1$) though the relationship between duration of haloperidol treatment and apomorphine response appeared less regular in the old mice.

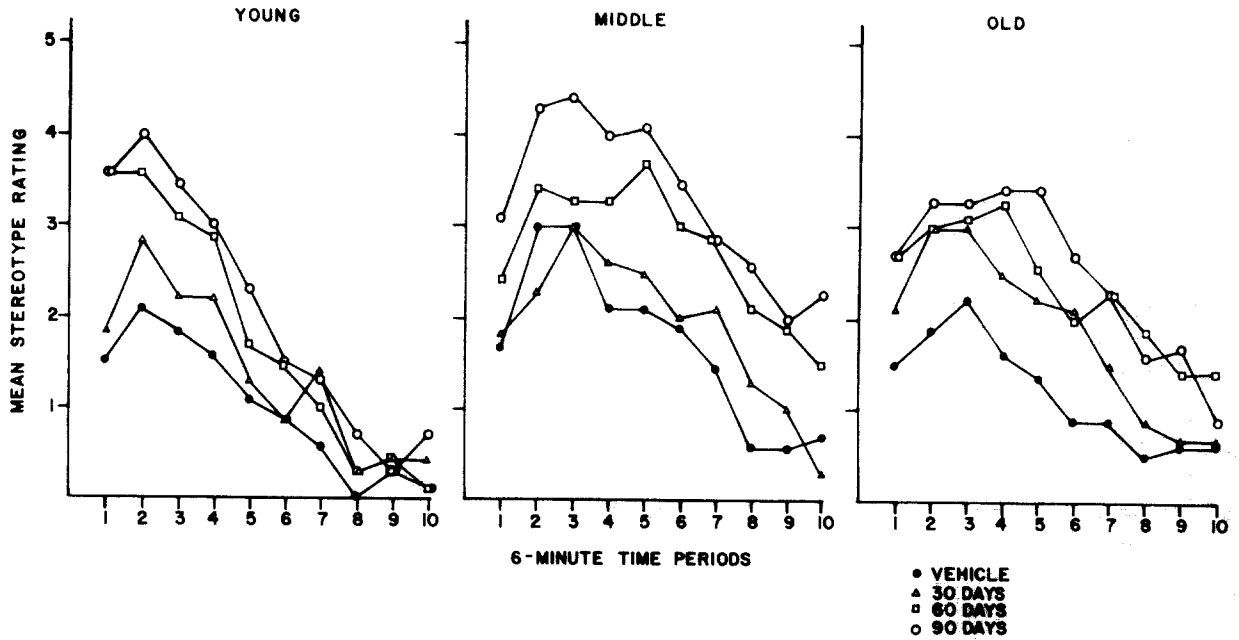

Fig. 1. Mean stereotype rating as a function of age and time after injection of 1.0 mg/kg apomorphine in mice treated with 2.5 mg/kg/day haloperidol for 0, 30, 60 or 90 days. Points represent means of 7–8 mice.

Stereotypic ratings increased between 6½ and 13 months and then decreased slightly in the older mice ($F = 4.01$; df = 2,73; $P < 0.025$).

Since the Age × Haloperidol Treatment interaction was not significant, the Haloperidol Duration effect was examined across ages and a highly linear relationship was obtained between duration of haloperidol and behavioral response to apomorphine over the 90 days of treatment. Trend analysis (orthogonal polynomials) on these data confirmed this with a highly significant linear component ($F = 18.57$; df = 1,73; $P < 0.001$) with no significant residual ($F < 1$).

Stereotype ratings first increased then waned with time ($F = 8.28$; df = 9,657; $P < 0.001$) as expected. A more prolonged time course in the middle- and old-aged mice was reflected in a significant Time × Age interaction ($F = 5.41$; df = 27,657; $P < 0.001$). The

TABLE I

Effects of age and haloperidol duration on [^{3}H]spiperone binding in C57BL/6J mice

Values are mean ± S.E.M. of the number of experiments in parentheses. Binding analysis was performed on striata from individual animals. B_{max} varied significantly with age and haloperidol treatment duration with no interaction. No reliable effects of either variable or their interaction were detected in K_d values. Overall $K_d = 98 \pm 5$. Haloperidol was administered at a dose of 2.5 mg/kg in the drinking water.

	Duration of treatment (days)			
	0	*30*	*60*	*90*
B_{max} (fmol/mg protein)				
Young	519 ± 24 (7)	626 ± 43 (7)	627 ± 28 (7)	717 ± 53 (6)
Middle	450 ± 32 (7)	453 ± 34 (7)	603 ± 34 (7)	642 ± 28 (6)
Old	386 ± 37 (8)	491 ± 45 (7)	502 ± 48 (7)	567 ± 48 (7)
K_d (pM)				
Young	116 ± 20	102 ± 16	82 ± 7	90 ± 10
Middle	86 ± 11	91 ± 11	99 ± 24	104 ± 23
Old	108 ± 29	96 ± 11	90 ± 7	95 ± 27

differential time course was statistically reliable between young and middle ($F = 7.45$; df = 9,657; $P < 0.001$), and young and old ($F = 4.33$; df = 9,657; $P < 0.001$) but not between middle and old ($F < 1$).

The absence of a significant Haloperidol Duration × Apomorphine Time Course interaction ($F < 1$) suggests strongly that the elevation of stereotype scores resulting from chronic haloperidol was not dependent upon altered apomorphine metabolism. Additionally, results of an analysis using only the maximum rating received by each animal yielded similar results, i.e. a linear relationship ($F = 8.41$; df = 1,64; $P < 0.01$) with no significant residual ($F < 1$). While this measure is not completely free of potential drug metabolism effects, it is certainly less confounded than total rating.

No significant blocks (session) effects were observed ($F = 1.59$; df = 6,66; $P > 0.05$).

[^{3}H]spiperone binding data

Results of [^{3}H]spiperone binding were similar to those for the behavioral data (Table I). B_{max} generally decreased with age, with the largest decrease occurring between 6½ and 13 months ($F = 24.78$; df = 2,63; $P < 0.001$) and increased with haloperidol duration ($F = 24.16$; df = 3,63; $P < 0.001$), but no interactions were significant ($F < 1$), indicating similar haloperidol response across age. Consistent with the behavioral data, a linear increase in [^{3}H]spiperone binding as a function of haloperidol duration ($F = 70.25$; df = 1,63; $P < 0.001$) was observed with no significant deviation from linearity ($F = 1.11$; df = 2,63; $P > 0.05$).

No reliable effects of age or treatment on the K_d were observed (Table I).

Relationship between behavior and binding

Since the current [^{3}H]spiperone binding assay is done on tissue from single animals rather than on pooled tissue, additional assessment could be made of the relationship between the behavioral and biochemical data.

Fig. 2 shows the relationship between the mean B_{max} for spiperone binding and mean total stereotype rating for all groups with error ellipses representing the S.E.M. on each of the variables. Two distinct linear relationships are apparent. The overall correlation coefficient between *means* of groups was 0.96, a figure consistent with the highly linear nature of the function. The middle-aged and old animals fall on the upper, and the young on the lower of two parallel lines (slopes = 0.0079, 0.0061 respectively; $F < 1$). Thus, the behavioral output as a function of receptor number is higher in the two older groups, this difference being primarily dependent upon the difference in apomorphine time course as discussed above.

Fig. 3 shows individual subject data for the behavioral and receptor binding data for all three ages. Since the block effect (assay runs) on the binding data was significant ($F = 10.7$; df = 6,63; $P < 0.001$), those data are shown as deviation from assay run mean. The relatively high degree of scatter in these data suggests that overall correlation in behavior/binding data are primarily dependent upon group differences with little or no relationship between the variables when assessed within haloperidol treatment groups. Moderate positive correlations were observed between total stereotype rating and receptor density in young- and middle-aged mice ($r = 0.52$ and 0.40 respectively) with a lower value in the oldest mice ($r = 0.18$). Even these modest correlations, however, were a result of between-group differences, as correlations of residuals around group means were substantially lower (young, $r = 0.08$; middle, $r = 0.02$; old, $r = -0.03$). Old mice appear much more variable, although the relatively small n per group precludes a meaningful statistical analysis of this effect.

Fig. 2. Relationship between striatal [^{3}H]spiperone binding (B_{max}) and stereotypic behavior in groups of C57BL/6J mice of different ages and receiving different durations of haloperidol treatment. Circles, 6½ months; squares, 13 months; triangles, 27–30 months.

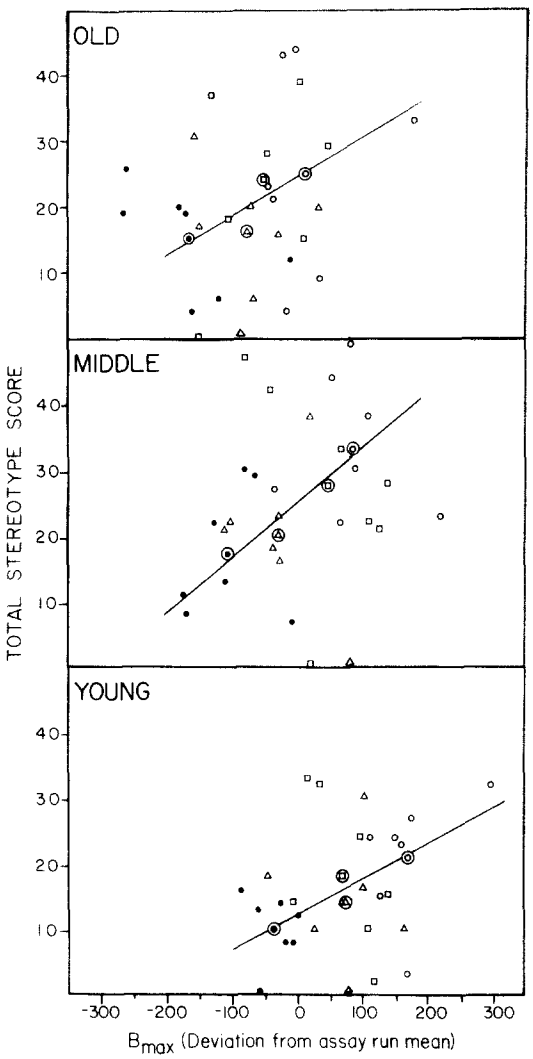

Fig. 3. Relationship between striatal [^{3}H]spiperone binding (B_{max}) and stereotypic behavior in individual C57BL/6J mice of different ages and receiving different durations of haloperidol treatment. Stereotypic behavior is expressed as total rating and binding as deviation from assay run mean. Solid circles, vehicle; solid triangles, 30 days; solid squares, 60 days; open circles, 90 days. Circled symbols represent group means.

Choline acetyltransferase activity

No alterations were detected in ChAT activity (Table II) as a function of either age ($F < 1$) or haloperidol duration ($F = 1.17$; df = 3,57; $P > 0.05$). The Age × Haloperidol interaction also failed to reach significance ($F < 1$).

DISCUSSION

Taken together, both binding and behavioral data from this experiment suggest that aging mice, in this case 27–30 months of age, retain the capacity for DA supersensitization to chronic haloperidol treatment. In contrast to our previously reported deficit in DA supersensitivity in old mice[53], we observed no statistically reliable differences in this effect between different age groups from 30 to 90 days. Unfortunately, we were not able to compare in the same experiment, aging animals which did and did not show supersensitization. Since our previous results were replicated in two substrains of C57BL/6 mice run a year apart, we feel it is unlikely that the original result was dependent upon sampling error. Instead, it is most likely that the age difference occurs relatively early in treatment, and may even have been underestimated at 21 days. It is interesting in this respect that tolerance to the cataleptic effect of neuroleptics occurs in two phases, a rapid phase over the first 5 days, and a more prolonged and continuing phase[2,17]. Old rodents may have deficits in the early phases of compensation for dopaminergic blockade. In addition, our previous study employed lower doses of haloperidol (1.2 mg/kg i.p. or 2.0 mg/kg in the drinking water) which, when combined with a shorter treatment protocol, probably resulted in an insufficient stimulus for receptor proliferation in the old mice.

We cannot determine from these data whether a stronger stimulus, e.g. a higher dose of neuroleptic over a shorter period of time, would also result in supersensitization. Lesions of the nigro-striatal pathway, however, do result in comparable supersensitization in young and old rats[33,34] and C57BL/6J mice[51]. In the latter study, we were unable to detect an age difference in the time course of the appearance of contralateral rotation following unilateral 6-OHDA-induced nigro-striatal lesion. It is likely that a sufficiently severe stimulus gives rise to a prompt behavioral supersensitization in the aging rodent. However, since neuroleptic- and lesion-induced supersensitization may be additive[67] they are probably not directly comparable.

In this experiment we found that both apomorphine-induced stereotypic behavior and [^{3}H]spiperone binding were linearly related to the duration of haloperidol treatment. DA supersensitization probably occurs very rapidly after lesion- or pharmacologically induced disruption of transmission in the nigro-striatal pathway. Contralateral rotation is detectable

TABLE II

Effects of age and haloperidol duration on striatal choline acetyltransferase activity

Values are mean ± S.E.M. of the number of experiments in parentheses. Data are expressed as nmol ACh formed/min/mg protein. No reliable effects of Age, Haloperidol duration, or their interaction were detected. Haloperidol was administered at a dose of 2.5 mg/kg in the drinking water.

	Duration of treatment (days)			
	0	*30*	*60*	*90*
Young	10.8 ± 3.5 (6)	11.4 ± 4.6 (6)	9.9 ± 3.3 (6)	7.4 ± 1.8 (5)
Middle	9.9 ± 3.5 (6)	9.7 ± 2.8 (5)	10.0 ± 7.0 (6)	8.4 ± 2.1 (5)
Old	10.6 ± 4.6 (7)	9.0 ± 4.2 (6)	7.7 ± 2.3 (5)	9.9 ± 3.3 (6)

within 2–3 days[47,68,73] after unilateral lesion and within 1–2 days[9,43] after neuroleptic administration. The increase in in vitro receptor binding is thought to take slightly longer, but is detectable within 1–3 weeks[7,15,68] and does not increase between 1 and 3 weeks[7]. The present data suggest that a continuing increase in both parameters occurs between 30 and 90 days, and are consistent with reports comparing 3 and 10 weeks haloperidol administration[16] and observations after very long (up to one year) treatment protocols[10,11,48]. Dewey and Fibiger[15] observed continuing increases in behavioral response to apomorphine, but not in [^{3}H]spiperone binding from 5 to 40 days treatment with pimozide. Continued alteration in striatal function over very long treatment periods may make age-related parameters difficult to assess, since the treatment period represents a significant portion of the life-span.

Many investigators[8,53,65,74] find aging rodents to be somewhat more sensitive than young to apomorphine. A possible interpretation of neuroleptic-induced supersensitization failure in old rodents is that receptor production and behavioral sensitivity are already at maximal levels, making it unlikely that drug treatment would enhance either. The results of the present study suggest that this is not so. Ability to show supersensitization is unaltered in aging mice given more prolonged treatment. The fall in basal levels of receptor protein with age may result from inadequate intraneuronal signalling for receptor production.

Similar deficits in supersensitization in the β-receptor system of old rats[24] have not been tested for duration of treatment effects. It is possible that given sufficiently prolonged or severe treatment aging animals would behave similarly to young.

In a more general context this result is similar to those from very different systems in which regulatory mechanisms have prolonged time-courses in aging animals. Finch et al.[20], for example, found that old C57BL/6J mice were delayed in the induction of hepatic tyrosine transaminase by cold environments.

The apomorphine time course data are suggestive of differential drug metabolism in the different aged mice. It is interesting in this respect, that the prolongation of time course is already present at middle age. Metabolism of several agents is known to be slowed with age[28,36,74], to the degree that behavioral responses to the drugs are altered[28,74]. Several investigators have reported higher stereotype ratings in old animals, and these data indirectly suggest that this is a result of altered drug clearance. If the middle-aged animals are used as controls, these data indicate that sensitivity may decline to some extent with age, consistent with the lowered receptor number. On the other hand, changes in other interacting systems may occur relatively early in life, to explain the higher ratings of the middle-aged and old animals. We have found[21], for example, that older animals are less sensitive to the cataleptogenic effects of pilocarpine, an effect that cannot be explained by slowed drug metabolism. Alterations in a cholinergically sensitive system might alter response to dopaminergic agonists, as has been demonstrated for the gnawing component of stereotypic behavior in mice[57].

Although D-2 sites have been implicated in apomorphine-induced stereotypic behavior, the lack of association of D-2 binding sites with the behavioral response observed in this study, when examined *within* groups, questions the direct causal relation between the two. Clearly, there is a good correlation

between in vivo potency for neuroleptic blockade of stereotypic behavior and in vitro blockade of D-2 binding[14,58]. However, in vivo studies, using an identical dose of apomorphine[38], showed only minor displacement of [^{3}H]spiperone by apomorphine and even larger doses of apomorphine did not displace specific [^{3}H]spiperone binding[30,40]. In addition, doses of sulpiride which block apomorphine-induced stereotypic behavior do not alter in vivo [^{3}H]spiperone binding[39]. Finally, behavioral supersensitivity precedes detectable increases in in vitro D-2 binding in both lesion[47,68] and chronic neuroleptic[32] paradigms. However, it is possible that increases in in vivo binding occur very rapidly[47]. Together with the data of the present study, these observations suggest that the correlation of D-2 sites and stereotypic behavior does not represent a simple one-to-one phenomenon.

Chronic haloperidol treatment elevates both parameters, but these may represent two different manifestations of chronic drug action, rather than increases in behavior being the direct result of elevated D-2 receptors. In addition, D-2 subtype number and regulation may be differentially affected by age[29]. The multiplicity of in vitro D-2 binding sites suggested by Scatchard analysis[1,6,50], drug displacement[31,55] or conversion of sites to high affinity agonist-binding sites[4,25] suggests that even if [^{3}H]spiperone B_{max} gives a total D-2 receptor estimation, it may not be the most precise variable to correlate with behavioral data. Agonist-preferring subtypes or spare receptors could obscure the correlational data.

Alternatively, the absence of a within group correlation between [^{3}H]spiperone binding and behavior may reflect the lack of precision in these measurements, relative to their biological variability. Experiments of this type are usually designed and run with the idea of minimizing within group variance. Highly controlled environmental and genetic characteristics should be expected to reduce the range of biological variability present in these experiments. Thus, age-, lesion-, or neuroleptic-induced changes may simply provide animals with sufficiently variable receptor number to detect this correlation. We observed an apparent greater variability of aging mice, with respect to apomorphine-induced behavior and a lower correlation between behavior and receptor binding. Heterogeneity of aging populations could be important determinants of drug response. This underlines the necessity for utilizing larger populations of aging animals on which a meaningful analysis of component sources of variance might be performed.

We found no alterations in choline acetyltransferase activity at any point in the haloperidol treatment. Activity of striatal cholinergic neurons is thought to be directly inhibited by DA or DA agonists, and blockade of DA receptors by neuroleptic drugs exerts characteristic effects on cholinergic biochemical parameters. Thus, acute neuroleptic treatment decreases acetylcholine (ACh) concentration in the striatum[66], and increases striatal efflux of ACh[66] and ACh turnover[67]. Tolerance to this effect develops: basal levels of ACh return to normal and further injections of the neuroleptic no longer influence striatal cholinergic activity[12,42,59,60]. Activity of ChAT is not thought to be an important variable in these changes[49] and these data are consistent with that view. Other investigators have found either no change[59] or decreases in ChAT activity[49] following chronic neuroleptic treatment. In the latter case, although total striatal ChAT was reduced by 42% after 6–8 weeks of treatment, no alteration in ACh formation or turnover was detected, suggesting that striatal ChAT is far in excess of that required to maintain normal cholinergic function. It should be noted that the detected loss of ChAT activity was measured in homogenates, while we measured the activity of the cytoplasmic enzyme. It is possible that major changes do occur in the membrane bound enzyme, although this accounts for only 15% of the total activity[22].

In general, these data suggest that age-related alterations in neuroleptic-induced DA supersensitization are sensitive to relatively small changes in drug administration protocols. It is most likely that duration of treatment and/or dose are particularly crucial, and deficits are most likely to be detected early in drug treatment at moderate doses.

We also failed to see any evidence of greater supersensitization at the longer duration treatments in old mice, which might account for the increased incidence of tardive dyskinesia in the elderly. Since there was no indication of asymptotic behavioral response or binding in these data, and since others have observed greater increases in ^{3}H-neuroleptic binding with 6 months to 1 year of treatment in rats, it is possible that aging animals do show enhanced supersensitivity with very prolonged treatment. It should be

noted, however, that the 90-day treatment in this group represents > 10% of the life-span. On the other hand, the greater occurrence of this syndrome in the elderly may result from deficiencies in opposing systems (e.g. cholinergic) or a predisposition caused by systems only distantly related to DA function.

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