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Effects of haloperidol on cholinergic striatal interneurons: relationship to oral dyskinesias

J. J. Kelley and **R. C. Roberts**

Maryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD, USA

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Summary. In a subset of rats, typical antipsychotic drugs (tAPD) produce oral dyskinesias called vacuous chewing movements (VCMs) that resemble tardive dyskinesia (TD), a behavioral side effect seen in a subset of people following tAPD treatment. Morphological changes within the striatum following tAPD have been correlated to VCMs in animal models. The cholinergic system has been implicated in expression of TD. To test the hypothesis that the striatal cholinergic system is perturbed after haloperidol treatment, rats were administered haloperidol for three weeks and tested for VCMs; the striata were then processed for the immunocytochemical localization of choline-acetyltransferase (ChAT). Neuronal density measures of ChAT-labeled neurons showed a 22% decrease in haloperidol-treated versus controls rats and a 37% reduction in the lateral portion of the striatum only in rats with VCMs. These findings further support evidence of the possible involvement of the cholinergic system and the ventrolateral striatum in VCMs, and possibly TD.

Keywords: Neuroleptics, tardive dyskinesia, acetylcholine, caudate, rat, VCM.

Introduction

Antipsychotic drugs (APD) are widely used in the treatment of schizophrenia as well as other psychotic disorders. Of these APDs, the traditional/typical antipsychotic drugs (tAPDS), such as haloperidol, are still frequently prescribed despite the acute and delayed extrapyramidal side effects including pseudoparkinsonism, acute dystonia and tardive dyskinesia (TD) that are frequently associated with their use (Klawans and Rubovits, 1972; Tarsy and Baldessarini, 1974, 1977; Jeste and Wyatt, 1979). TD, characterized by involuntary movements predominantly in the orofacial region, develops in a subset of patients (20 to 40%) after several months or years of treatment with tAPDs (Klawans and Rubovits, 1972; Tarsy and Baldessarini, 1974, 1977; Jeste and Wyatt, 1979). TD symptoms often

present long after treatment has begun and persist in over one-half of patients after cessation of tAPDs (Klawans and Rubovits, 1972; Tarsy and Baldessarini, 1974, 1977; Jeste and Wyatt, 1979), suggesting that there may be long-term morphological or functional changes in the brain associated with the symptoms (Meshul et al., 1992, 1994).

Animal models are extensively used in order to study the morphological or functional changes that may occur in the human brain following APD treatment. Moreover, APDs have been administered to experimental animals in numerous studies to clarify whether the onset, cessation, and degree of motor side effects are correlated temporally with functional or morphological brain changes. Administration of tAPDs to experimental animals results in development of a behavioral sequelae called vacuous chewing movements (VCMs), resembling clinical TD (Clow et al., 1980; Iverson et al., 1980; Tamminga et al., 1990; Waddington, 1990; Andreassen and Jorgenson, 2000) and often times a resting tremor, resembling that which is seen in pseudoparkinsonism patients. VCMs develop in a subset of rats administered tAPDs, reverse slowly (Waddington et al., 1982; Mithani et al., 1987; Tamminga et al., 1990; Waddington, 1990) and often persist well after withdrawal of APD (Egan et al., 1996; Levinson et al., 1998). ''*Tardive*'' animal VCM studies have been historically characterized as clinical models for chronic or long-term neuroleptic treatment, while acutely evoked VCMs have been thought to be the clinical equivalent of acute dystonia and pseudoparkinsonium tremor (Salamone et al., 1998; Egan et al., 1996).

There are several hypotheses regarding the mechanisms which underlie TD (Clow et al., 1980; Gunne et al., 1983; Fibiger and Lloyd, 1984; De Keyser, 1991; Gunne and Andren, 1993; See and Chapman, 1994; Post, 1980; Turrone et al., 2003), including loss of striatal cholinergic interneurons (reviewed by Miller and Chouinard, 1993). Striatal acetylcholine release is modulated by nigrostriatal dopaminergic function (Sanvarinda et al., 1998; Mizukawa et al., 1987). Dopaminergic afferents project throughout the striatum and terminate on cholinergic interneurons and projection neurons (Kawaguchi, 1995). Cholinergic interneurons, which comprise only 1–2% of the striatum, have extensive collaterals that create very dense local innervation (DiFiglia et al., 1976). Cholinergic interneurons form symmetric synapses (Phelps et al., 1985; DiFiglia and Carey, 1986; DiFiglia, 1987), and previous research from our laboratory has revealed a loss of striatal symmetric synapses, typical of inhibitory neurotransmission, only in the subset of rats with oral dyskinesias (Roberts et al., 1995; Roberts and Lapidus, 2003). This loss of inhibitory control in the striatum could be involved in VCMs observed in these rats, as the striatum has been established as a site involved in movement. An investigation by Grimm et al. (2001) showed a decrease in the number of cholinergic interneurons in rats administered haloperidol for six months. The purpose of the present study was to determine if the number of cholinergic neurons in the striatum was affected by haloperidol at the three-week time point, if there was a regional selectivity of this effect and if the effect was confined to rats with VCMs. This work has been presented in preliminary form (Kelley and Roberts, 2000).

Methods

Drug treatment

Two sets of Sprague-Dawley (Harlan) albino rats, treated identically but at different times, were used in this study. Rats were organized into two drug treatment groups that were used for the quantification of ChAT-immunolabeled neurons or cresyl violet stained neurons. Treatment groups consisted of: 1) a haloperidol-group ($n = 30$) administered a solution of 2.5 mg of haloperidol/100 ml water to drink, providing approximately 1.5 mg/kg/rat/day; and 2) a control group ($n = 20$) given water.

Haloperidol was purchased from Sigma (H1512) and dissolved in glacial acetic acid to form a stock solution at a pH between 5.5 and 6.0 and refrigerated, for no longer than two weeks, prior to use. The stock solution was diluted with tap water to give a haloperidol concentration of 0.025 mg/ml at an approximate pH of 7 and administered in dark amber drinking bottles. The chosen dosage has been shown to mimic blood serum levels seen in the clinical population (Van Putten et al., 1985; Hashimoto et al., 1998). This method of drug delivery has been established as a reliable method (Tamminga et al., 1990) and is in keeping with our previously detailed research methods (Roberts et al., 1995). Rats were treated for a period of 21 days. During the course of the study all animals were treated according to institutional guidelines.

Behavioral testing

Rats, initially weighing 175–200 g (P46–49), were allowed to accommodate to laboratory conditions for one week, before a baseline VCM rating was performed, prior to drug administration. By study end, rats were individually assessed a total of three to five times for VCMs. Scores were assessed by a rater blind to the treatment groups. At study end, three weeks later, two VCM ratings were performed and averaged for a final score. Rats were divided, based on their average final score, into two behavioral groups: Low VCM (<8 VCMs/5 min) or High VCM ($\geq$8 VCMs/5 min) (Fig. 1). If the two final VCM scores were not within the same VCM group, either Low or High, then two more ratings were performed and the four scores were averaged for a final score. The score of eight has been determined by mixture analysis to be the pivotal point between Low and High VCMs (Hashimoto et al., 1998).

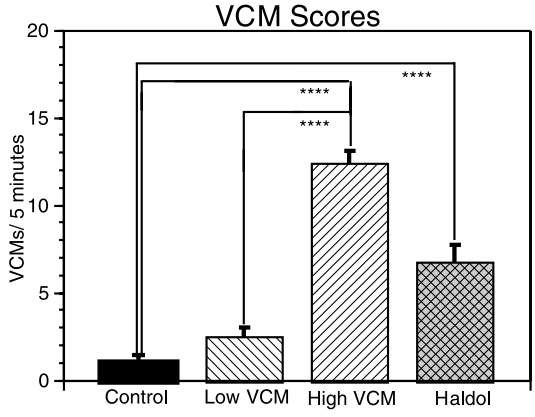

Fig. 1. Graph illustrating the behavioral scores at study end for control ($n = 20$), Low VCM rats, ($n = 17$), High VCM rats ($n = 13$), and Haldol ($n = 30$), combined scores for Low and High VCM rats. The VCM scores of the Haldol treated group were shown to be statistically higher (unparied t-tests) than that of the control. VCM scores of the High VCM group were significantly higher (Fisher's PLSD) than that of the Low VCM group and that of the controls. Error bars indicate standard error (**** $p < 0.001$)

Tissue preparation

At study end, all rats were deeply anesthetized with a mixture of ketamine/xylazine (5 mg ketamine + 1 mg xylazine/100 mg body weight). Rats were perfused in sets of three (one control, one Low VCM, and one High VCM) with saline (100 ml) followed by 4% paraformaldehyde (500 ml) in 0.1 M phosphate buffer, pH 7.4 (PB) over the course of four days. Brains were sectioned with a vibratome in the coronal plane at 100 μm, beginning at the most rostral point of the striatum (Bregma 2.2 mm) and extending caudally until the appearance of the dentate gyrus (Bregma −3.3 mm). A series of sections (300 μm apart) were simultaneously processed for immunocytochemistry. Briefly tissue was rinsed in phosphate buffered-saline (PBS), rinsed 15 minutes in 1% sodium borohydride, thoroughly rinsed in PBS, followed by rinses of 1.5% hydrogen peroxide and then PBS, pre-blocked in 3% goat serum and 0.3% Triton-X and incubated in rabbit anti-choline acetyltransferase (Chemicon-Temecula, California) at a dilution of 1:5000 in 1% goat serum and 0.3% Triton-X for 60 hours. Sections were further processed with avidin-biotin complex (Vector Laboratories), reacted with diaminobenzidine (Sigma), mounted on slides, dehydrated through alcohol, and coverslipped prior to viewing. Another series of sections (300 μm apart) were stained with cresyl violet, dehydrated through a series of alcohol dilutions and coverslipped.

Neuron counts and area measurement

Five coronal sections, from the series of sections taken throughout the rostral-caudal extent of the striatum, were identified by similar morphological characteristics for each rat and assigned a corresponding section (SXN) number (SXNs 1–5) (Fig. 2). Within each of the five sections, cell counts were consistently taken from five specific regional areas (Dorsal, Medial, Central, Lateral, and Ventral) from both the right and left hemispheres (totaling 50 areas/rat @ 500 μm^2 each).

ChAT counts

ChAT-immunoreactive counts were analyzed in three ways: 1) *total neuronal counts* averaging all 50 areas within each rat per treatment group, 2) *rostral-caudal comparison* analyzing total neuronal counts within each SXN(1–5) from combined regional areas of each rat per treatment group and 3) *regional striatal comparison* analyzing total neurons within each striatal region, Dorsal, Medial, Central, Lateral, and Ventral from each SXN across R-C extent (except SXN 5) for each rat per treatment group. Section 5 was omitted from the *regional striatal comparison* because the regional area were not equivalent to the same regional areas found in SXNs 1–4, due to the dramatic change in the shape of the caudal striatum from previous sections. Regional areas were counted in an ocular grid at a magnification of 20X equaling 500 μm^2. The criteria for labeled ChAT cells were: 1) somal labeling with a visible nucleus and/or dendritic process and 2) a cell body size $\geq$15 μm × $\geq$20 μm (minor × major) determined by the measurement scale within the ocular grid.

Nissl counts

Nissl counts were obtained by averaging all 50 areas within each rat per treatment group. Nissl-stained large aspiny neurons were markedly more difficult to count than ChAT-labeled cells, as they were obscured by the abundance of Nissl stained medium spiny neurons; therefore to increase visibility four areas were counted at a magnification of 40X in an area equaling 250 μm^2 and added to equal 500 μm^2 for each regional area. Nissl stained large aspiny neurons were identified by a cell body size $\geq$15 μm × $\geq$20 μm (minor × major) determined by the measurement scale within the ocular grid.

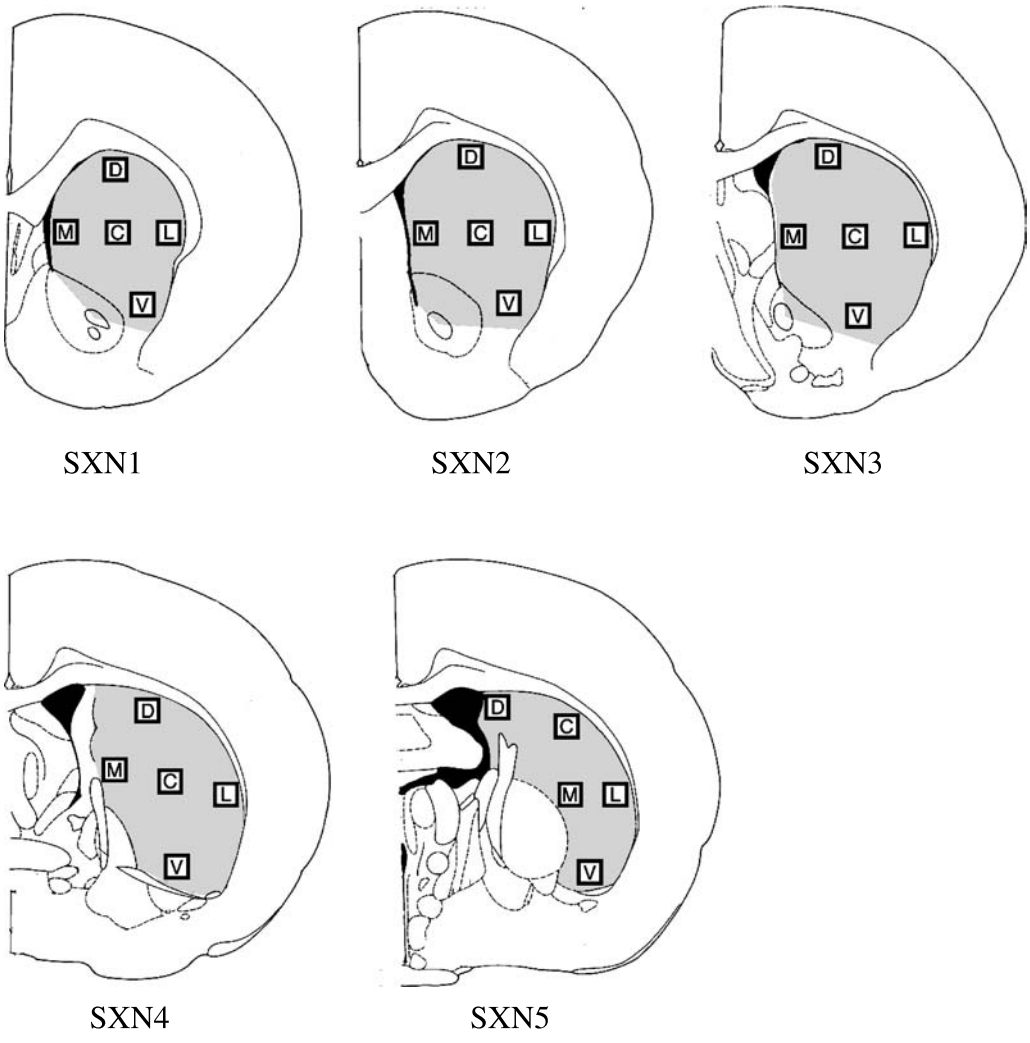

Fig. 2. Schematic illustration showing representative sections from the rostrocaudal extent of striatum representing the approximate area from which the measurements were taken. Each rostral to caudal section SXN1-5 represents Bregma areas 1.6 mm, 1.0 mm, 0.48 mm, 0.3 mm, and −0.92 mm, respectively. The extent of the striatum is large and the shape somewhat irregular. For area analysis of the striatum the gray shaded regions were measured using NeuroZoom. The placement of the five boxes represents the location of the 500 μm^2 area where the ChAT-labeled and Nissl stained neurons were counted. Regions labeled D, M, L, C, V represent the respective dorsal, medial, lateral, central, and ventral regions

Area measurements

The same five sections analyzed for ChAT-labeled neurons were measured for striatal area using NeuroZOOM. Striatal boundaries were defined as shown in Fig. 2 and follow commonly characterized striatal boundaries (Roberts, 2001; Roberts et al., 2002).

Data analysis and statistics

All statistical analysis was performed using StatView on a Macintosh platform. To examine the effects of haloperidol, data was organized into two groups either a control or drug group and

analyzed using unpaired t-tests. For behavioral analysis, data was organized into three categories: control, Low VCM, and High VCM and analyzed by one-way ANOVAs followed by Fisher's post-hoc multiple comparisons (PLSDs) for comparison of control, Low VCM, and High VCM group.

Results

VCMs

The mean VCM scores for all rats used in this study are shown in Fig. 1. After 3 weeks of haloperidol treatment approximately half of the group of rats had High VCM scores. The VCM scores from the rats in the haloperidol-treated group were significantly higher ($p < 0.0001$) than those of the control group. VCM scores also differed significantly across control, Low VCM and High VCM rats ($F(2,47) = 104.46$, $p < 0.0001$). The VCM scores from the High VCM group were significantly higher than the scores of the Low VCM group ($p < 0.0001$), as well as the scores of the control group ($p < 0.0001$). There was no significant difference between the scores in the Low VCM group vs. controls.

Striatal area

Analysis showed no significant difference in area (mm^2) of the striatum between the control group (9.01 ± 0.97) and the haloperidol-treated group (9.55 ± 0.56). There was no significant difference in overall area of the striatum across control, Low VCM (9.56 ± 0.81) or High VCM (9.50 ± 0.73) animals. Moreover, comparison of the controls, the Low VCM group and the High VCM group showed no significant difference in striatal area.

Qualitative observations

All ChAT-immunoreactive cells presented large somata and many but not all neurons presented extensive dendritic processes (Fig. 3). Brief blind-qualitative analysis, conducted independently by two different observers, suggested that only control rats presented the most extensive and far reaching labeling within the dendritic processes and the most robust staining of a magnitude not noted in the Haloperidol-treated rats. Nissl stained large neurons in the haloperidol treated rats appeared morphologically identical to those of the control rats (Fig. 4).

Overall neuronal density

The density of ChAT-labeled neurons were significantly reduced by 22% ($p < 0.05$) among rats administered haloperidol in comparison to controls (Fig. 5). The density of ChAT-labeled neurons was significantly different among the three behavioral groups ($F(2,24) = 4.07$, $p < 0.05$). There was no significant difference in the density of labeled cells between the Low VCM group and the High VCM group. However, High VCM rats showed a 29% decrease in the density of ChAT-immunoreactive cells in comparison to controls, ($p < 0.01$).

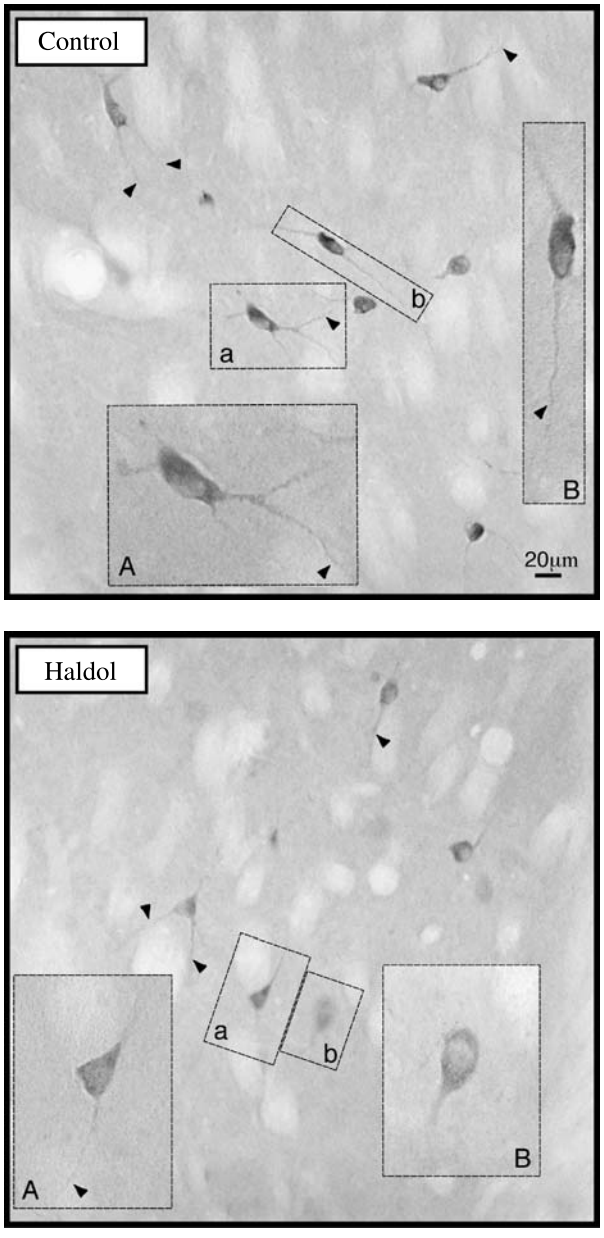

Fig. 3. Light micrographs showing ChAT-labeled large neurons taken from the lateral region of the striatum at 20× with a digital camera. The solid black outline surrounding the micrographs represents the counting frame of 500 μm^2. Arrow heads indicate labeled dendrites. Within each micrograph the image outlined with the black dotted line box is shown at higher magnification (A or B). Due to the thickness of the section, not all of the labeled cells are in the same focal plane. The top micrograph is taken from a control rat and represents the typical staining of large aspiny ChAT-immunoreactive cells found in this group. Note the extensive labeling within the dendritic arbor. The bottom micrograph is taken from a rat within the Haldol group and represents the best, but not the average, staining of large ChAT-immunoreactive cells (see Results). Note that the ChAT-immunoreactivity observed in these cells is slightly less robust than the ChAT-immunoreactivity observed in the control micrograph. Scale bar represents 20 μm

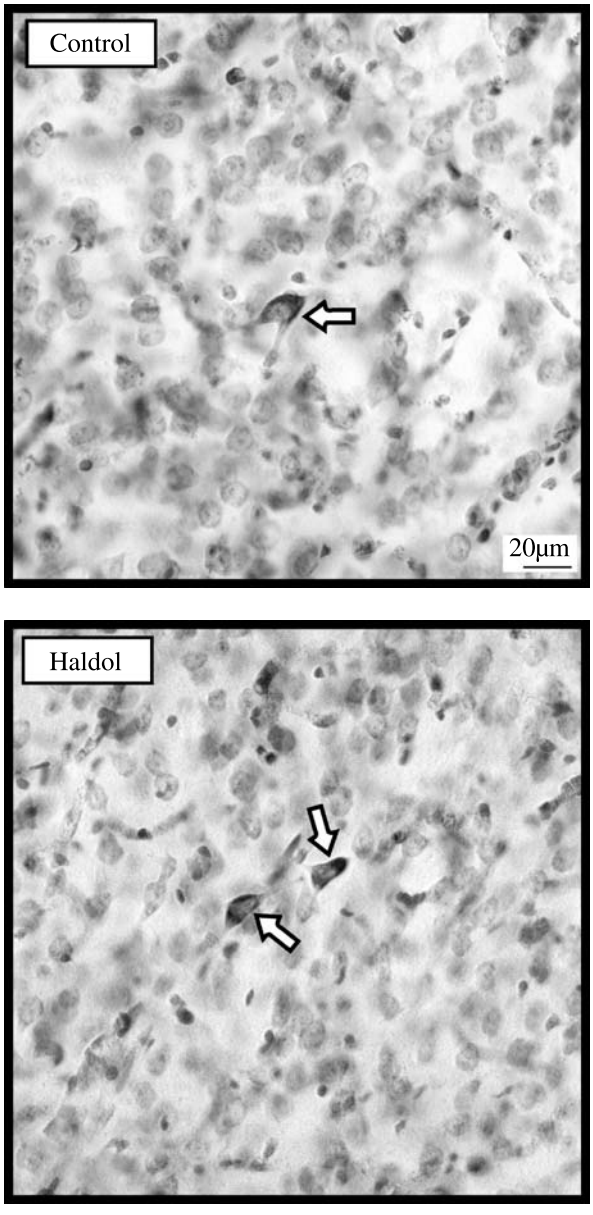

Fig. 4. Light micrographs taken at 40× with a digital camera representing the Nissl staining observed in either the Control (top) or Haldol (bottom) group. The solid black outline surrounding the top and bottom micrographs represents the counting frame of 250 μm^2. Note that there is no observable difference in cell morphology between the control and Haldol group of the large neurons (arrow), including no gross signs of neurodegeneration. Further, note the abundance of medium spiny neurons surrounding the large aspiny neurons. Scale bar represents 20 μm

No overall difference existed in the density of Nissl stained large aspiny neurons in the striatal areas examined, between rats administered haloperidol (15.87 ± 3.7) and rats in the control group (15.28 ± 4.5). Further statistical analysis (ANOVA) showed no overall difference between the density of Nissl stained large aspiny neurons in the striatal areas examined among the control,

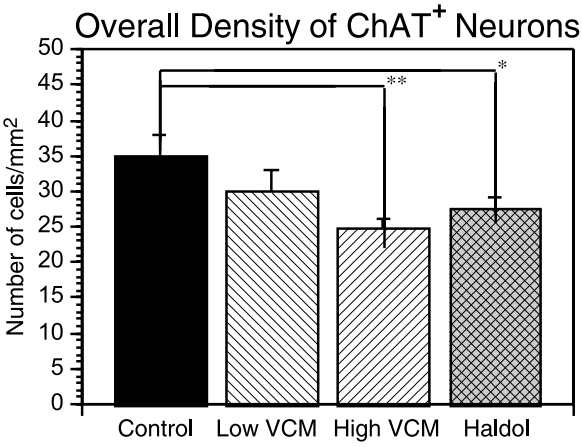

Fig. 5. Graph illustrating the overall mean density of ChAT-labeled neurons counted in each group from each of the five striatal regions (D, M, C, L, V) within each of the five rostral-caudal sections sampled. The number of ChAT-labeled cells is significantly decreased in the Haldol treated group in comparison to controls with a greater and preferential decrease seen in the High VCM group. Error bars indicate standard error. Significant differences of either unpaired t-tests (control vs Haldol) or Fisher's PLSDs (control vs High or Low VCM) are shown (* $p<0.05$; ** $p<0.01$)

Low VCM rats (15.33 ± 4.7) and High VCM rats (16.54 ± 2.1). Since there were no differences in density of cresyl violet stained cells, rostrocaudal and regional analysis of Nissl sections was not performed.

Rostral-caudal comparison

The density of ChAT-labeled cells was affected by haloperidol differently across the rostrocaudal extent of the striatum (Fig. 6). Although the density of ChAT-labeled cells in the haloperidol treated animals at Bregma 1.6 mm (SXN 1) showed evidence of a decreasing trend ($p = 0.054$) in comparison to controls, a significant decrease in the density of ChAT-labeled neurons in the haloperidol-administered group compared to controls was observed specifically in the areas caudal to and including Bregma 0.50 mm (an average decrease of 26%). The density of ChAT-labeled neurons was significantly different (ANOVA) among the three groups in SXN 4 ($F(2,24) = 7.00$, $p<0.01$) and SXN 5 ($F(2,22) = 4.01$, $p<0.05$). Fisher's PLSD revealed that the density of ChAT-immunoreactive cells within the High VCM group was significantly less in comparison to the control group in all but SXN 2. A significant difference was observed between Low and High VCM rats only in SXN 4 ($p<0.05$). There was no significant difference in the density of ChAT-labeled cells in the Low VCM group compared to controls in any section.

Regional comparison

Heterogeneous differences in the density of ChAT-labeled cells were observed among striatal regions identified as dorsal, lateral, central, medial, or ventral. The density of ChAT-labeled cells was decreased significantly ($p<0.01$) in the lateral striatum of haloperidol treated rats as compared to controls (Fig. 7).

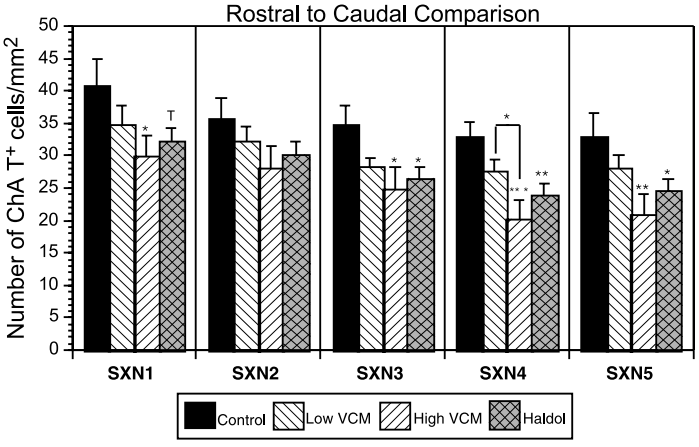

Fig. 6. Graph illustrating the mean number of ChAT-labeled neurons counted in each group for each of the five rostral to caudal sections illustrated in Fig. 2. All five D, M, C, L, V regions were averaged per section. SXNs 3, 4, and 5 show an overall decrease of 35% in ChAT-labeled neurons within the High VCM group vs control. The most profound decrease was seen at SXN4 (Bregma 0.3 mm), wherein the High VCM group showed a 39% decrease from control rats and the only significant decrease in the number of ChAT labeled cells in comparison to Low VCM rats. Error bars indicate standard error. Significant differences of either unpaired t-tests (control vs Haldol) or Fisher's PLSD's (control vs High or Low VCM) are shown (T, $p < 0.06$; * $p < 0.05$; ** $p < 0.01$)

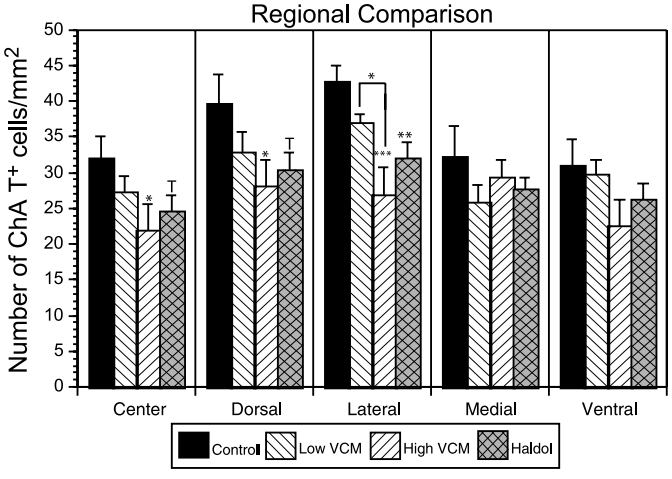

Fig. 7. Graph illustrating the mean number of ChAT-labeled neurons counted in each group. The average was calculated for each striatal region from each SXN across the R-C extent (except SXN 5) for each rat group. Note, the number of ChAT-labeled cells was decreased significantly in the lateral striatum of haloperidol treated rats when compared to controls. The lateral, dorsal and central striatum of the High VCM rats had significantly less ChAT-immunostained cells in comparison to controls rats. Note only the lateral striatum showed a significant decrease in the number of the ChAT-labeled neurons between Low and High VCM rats. Error bars indicate standard error. Significant differences of either unpaired t-tests (control vs Haldol) or Fisher's PLSD's (control vs High or Low VCM) are (T, $p < 0.06$; * $p < 0.05$; ** $p \leq 0.01$; *** $p < 0.001$)

Comparison of the central and dorsal striatal regions of haloperidol-treated animals showed evidence of a trend ($p < .06$) towards a decrease in the density of ChAT-labeled cells in comparison to the respective central and dorsal striatal areas in controls. No differences were observed among the behavioral groups (ANOVA) in the medial, central, ventral, or dorsal regions of the striatum. However, an overall behavioral difference was found in the lateral striatum ($F(2,24) = 8.46$, $p < 0.01$) wherein the High VCM rats had significantly less ChAT-immunostained cells in comparison to both controls ($p < 0.001$) and Low VCM ($p = 0.0158$) rats. As well, significantly less ($p < 0.05$) ChAT-immunostained cells were found in the dorsal and central regions of High VCM rats in comparison to controls.

Discussion

The results of the present study: 1) confirm and extend previous studies indicating that the striatal cholinergic system is affected by haloperidol treatment at three weeks of treatment; 2) show that there is a regional selectivity of this effect; and 3) indicate that the effect is more profound in rats with VCMs. This significant reduction in the density of large aspiny neurons expressing ChAT may have profound effects on the neuronal transmission dependent on these neurons. The observation that a decrease in ChAT-labeled cells occurs early in treatment and preferentially in animals with VCMs, is consistent with a causal role for a loss of cholinergic tone and the expression of VCMs.

An unbiased stereological investigation was not used in this study. As previously detailed, the preface of this study was contingent on the investigation of the density of cells within the anatomically delineated parts of the striatum (dorsal, ventral, lateral, medial, and central). Studies with similar goals as our own (Grimm et al., 2001; Holt et al., 1999), have not used three-dimensional stereology and for comparative purposes we too chose to use two-dimensional counting techniques. Thus, we believe that our chosen methodology has yielded accurate and interpretable results identifying region specific differences in neuronal density (Benes and Lange, 2001).

A slight increase in striatal size (<10%) in rats following APD administration (Chakos et al., 1994) and in individuals suffering from schizophrenia (Chakos et al., 1998) could lead to some skepticism as to whether any decrease in ChAT-labeled neuronal density within the striatum could be accounted for by increased striatal size. However within the present study, as well as our previous studies, no significant changes were found in the striatal size of rats following haloperidol treatment (Lapidus et al., 1998; Roberts, 2001). A large increase in striatal size would have to occur to account for the 22–39% decrease in neuronal density observed in the present study. Moreover, the Nissl material within the present study showed a normal number of large neurons in the drug treated rats. Therefore, our counts of ChAT-immunoreactive neurons are most likely an accurate representation of neuronal density that can not be discredited by a marginal increase in striatal volume.

A decrease in the density of ChAT-immunoreactive neurons may indicate either down-regulation of ChAT production or cell death of some cholinergic

interneurons. However, no evidence of neurodegenerative pyknosis of ChAT-immunoreactive or large neurons in Nissl material was observed in this study. Furthermore, analysis of the Nissl material in the present study showed no difference in the density of large neurons between the control, Low VCM, High VCM or the combined haloperidol treated group. Therefore it could be surmised, after three weeks of treatment, that the decrease in density of ChAT-immunoreactive cells is not due to neuronal death, but to downregulation of ChAT in a proportion of those cells. That said, this finding does not negate the possibility that a longer time course of drug administration may potentially yield neurodegeneration, as striatal neurodegenerative processes have been previously observed in cholinergic cell bodies following haloperidol treatment at longer treatment intervals (Mahadik et al., 1988).

The regional examination of the density of ChAT-immunoreactive neurons revealed specific differences in neuronal density. Compared to controls, haloperidol treatment resulted in a decrease (26%) in the number of ChAT-immunoreactive neurons starting at and caudal to Bregma 0.50 mm with the decrease being more profound within the High VCM group (29–39%). This larger affect at caudal levels may be due to the topographical organization of the nigrostriatal pathway (Parent and Hazrati, 1995), as similar abnormalities have been observed in dopaminergic neurons within the substantia nigra, particularly at caudal levels. For instance, two studies showed that a proportion of dopaminergic neurons in the substantia nigra of rats become devoid of tyrosine hydroxylase (TH) immunoreactivity following three weeks (Levinson et al., 1998) and/or six months (Kelley et al., 1999) of haloperidol treatment. In a preliminary study (Kelley et al., 1999), the rostrocaudal extent of the SN was examined and the loss of TH-labeled neurons was more prevalent in caudal regions of the SN.

Our regional quantification also revealed a decrease in the density of ChAT-labeled cells in the lateral region in the Haldol treated group (26%) and High VCM group (37%) in comparison to the control group. The significant difference in the decrease in labeled cells between the Low and High VCM groups (28%), suggests that the decrease in cholinergic transmission in the lateral striatum may be associated with VCMs. As noted previously, the ventrolateral portion of the striatum is implicated in orofacial movements (Delfs and Kelley, 1990; Hasegawa et al., 2001; Kelley and Delfs, 1994; Kelley et al., 1988, 1989a, b; Pisa, 1988; Salamone et al., 1990), as microinjections of either cholinomimetics or dopaminergic depleting agents within the VLS have resulted in the emergence of VCMs (Kelley et al., 1989a; Jicha and Salamone, 1991).

Given our results that there are fewer ChAT-immunoreactive neurons in rats with VCMs, it may seem peculiar that direct addition of acetylcholine by microinjection, rather than depletion of acetylcholine in this region, elicits acute VCMs. However, *chronic* neuroleptic-induced oral dyskinesias, as opposed to acute stimulation, have been shown to be suppressed by cholinergic agonists, suggesting a compromise of cholinergic transmission in chronically induced TD/VCMs (Tamminga et al., 1977). While past literature suggests that VCMs may be produced from decreased or upregulated cholinergic transmission, morphological and neurochemical markers have identified other differ-

ences between acute and chronic VCMs (Egan et al., 1996). Thus, the seemingly contradictory results of our study may be explained, in part, by the brain's differential response to APDs depending on the length of treatment (Uranova et al., 1991; Roberts et al., 1995; Meredith et al., 2000).

The results of the present study are consistent with similar studies in the literature. For example, Grimm et al. (2001), observed a significant decrease in ChAT-labeled neurons in the ventrolateral striatum in rats after six months of haloperidol treatment and one month withdrawal. The magnitude of this decrease approximated from visual inspection of Fig. 2 is about 25%. Mahadik et al. (1988) examined biochemical levels of ChAT in rats treated with haloperidol and found a 26% reduction in ChAT activity in rats treated for 40 days, lighter staining in labeled cells and morphological alterations not seen in the present study. Importantly, the results of Mahadik et al. (1988) show a reversal in the activity of cholinergic enzymes between 7 and 40 days, ending with evidence of a cholinergic deficiency after several weeks of treatment.

The cholinergic system has been implicated in the pathophysiology of schizophrenia (Dean et al., 2000; Tandon et al., 1999). Investigation of the striatum in the brains of individuals suffering from schizophrenia has shown a decrease in number of cells labeled with ChAT (Holt et al., 1999), consistent with reports of a decrease in ChAT levels within the striatum (Bird et al., 1977). In the study by Holt et al. (1999), the loss of ChAT immunoreactivity was interpreted to be a possible neuropathological correlate of schizophrenia, rather than a consequence of medication, because two of the ten post-mortem cases, showing the most marked decrease in immunoreactivity, were from schizophrenics that had been without neuroleptics for at least one month. Despite, the marked decrease in density of cholinergic neurons found by Holt et al. (1999), the author reiterated that none of the schizophrenic cases, used in the analysis, were from entirely neuroleptic-naïve patients and further acknowledged that previous antipsychotic treatment could have been involved in the observed decrease. Interestingly, the 24% decrease in number of ChAT-immunoreactive neurons in the striatum that Holt et al. (1999) reported (percentages calculated from density measurements reported in Table II) is remarkably similar to that which was observed in ours and other studies (Grimm et al., 2001; Mahadik et al., 1988). Therefore, given the results of this study, as well as others (Grimm et al., 2001; Mahadik et al., 1988), it is plausible that the decreased number of striatal neurons reported by Holt et al. (1999) in the subjects with schizophrenia may indeed have been caused by APD treatment.

In addition to the putative role of acetylcholine in the pathophysiology of tardive dyskinesias, acetylcholine may play a role in cognitive deficits that have been observed in patients taking either anticholinergics or tAPDs (Waddington, 1990). For example, a negative correlation has been observed between cognitive impairment and ChAT activity in postmortem studies of schizophrenic subjects (Powchik et al., 1998), as well as a positive correlation between cognitive impairment and tardive dyskinesias (Waddington, 1990). These findings thereby suggest a keen involvement of acetylcholine in both the cognitive impairments and tardive dyskinesia symptomatology observed in these medicated patient populations. Understanding the cellular and or morphological

changes resulting from APD therapy may give insight into better therapeutic treatments as well as a future interpretation of results obtained from investigations of human post-mortem brains.

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Authors' address: J. J. Kelley, Maryland Psychiatric Research Center, P.O. Box 21247, Baltimore, MD 21228, USA, e-mail: jkelley@mprc.umaryland.edu