Effect of Chronic Olanzapine Treatment on Striatal Synaptic Organization

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KEY WORDS caudate; antipsychotics; haloperidol; synapses; tardive dyskinesia; vacuous chewing movements

ABSTRACT Our previous work has shown that chronic haloperidol treatment decreases striatal symmetric synapses preferentially in rats which develop oral dyskinesias (vacuous chewing movements (VCMs)). The present experiment tests the hypothesis that olanzapine, which does not cause dyskinesia in humans or rats, would not cause the ultrastructural changes produced by haloperidol. After 6 months of treatment, VCM scores for the olanzapine group (5.1 ± 4.5) were similar to those of controls (5.2 ± 3.9), whereas rats in the haloperidol group were either nondyskinetic (4.3 ± 2.2) or dyskinetic (16.9 ± 6.7). The volume of the striatum (mm^3), did not differ among the groups: control, 37.5 ± 4.7; olanzapine, 36.4 ± 4.3; haloperidol, nondyskinetic, 40.5 ± 6.3; haloperidol, dyskinetic, 36.6 ± 5.9. Synaptic density (per 1 μm^3), obtained from the central region of the striatum, did not differ between the olanzapine (0.699 ± 0.146) and control groups (0.652 ± 0.108). The number of asymmetric synapses in the olanzapine group (0.624 ± 0.136) was also similar to that of controls (0.550 ± 0.090). The number of symmetric synapses in the olanzapine group (0.074 ± 0.032) was not significantly different from that of controls (0.096 ± 0.043). Thus, olanzapine, in contrast to haloperidol, did not produce dyskinesias or synapse loss. These results strengthen the correlation between the expression of VCMs and striatal synaptic changes and indicate that olanzapine has fewer behavioral and anatomical side effects than does haloperidol. **Synapse 39:8–15, 2001.**

INTRODUCTION

Neuroleptics given to rats provide an experimental model to study both the general effects of antipsychotics on the brain (Benes et al., 1985a,b; Meshul and Casey, 1989; Meshul and Tan, 1994; Meshul et al., 1992; Roberts et al., 1995; Uranova et al., 1991) and the behavioral, pharmacological, and anatomical correlates of neuroleptic-induced movement disorders (Burt et al., 1977; Clow et al., 1980; Gunne and Haggstrom, 1983; Iverson et al., 1980; Roberts et al., 1995; Tamminga et al., 1990; Waddington, 1990). Typical neuroleptics given chronically to rats induce behavioral sequelae which mimic tardive dyskinesia in several ways. Numerous studies have examined neuroleptic-induced changes in striatal anatomy. For instance, chronic haloperidol treatment in rats and humans produces a slight enlargement in the striatum (Chakos et al., 1994, 1998), whereas atypical antipsychotics do not have this effect. In experimental animals, changes in synaptic organization occur after either subchronic or chronic treatment (Benes et al., 1985a,b; Meshul and Casey, 1989; Meshul and Tan, 1994; Meshul et al., 1992; Uranova et al., 1991). Moreover, particular anatomical changes have been correlated with oral dyskinesias (Egan et al., 1994; Kelley et al., 1997; Roberts et al., 1995). Our previous work has shown that chronic treatment with haloperidol decreases overall striatal synaptic density, and that symmetric synapses are lost only in the subset of rats with oral dyskinesias (vacuous chewing movements (VCMs)) (Roberts et al., 1995).

Atypical antipsychotics, such as clozapine, olanzapine, and sertindole, have been used in experimental animals to assess the incidence of VCMs (Gao et al., 1998; Kakigi et al., 1995) or to look at anatomical correlates of drug treatment (Meshul and Casey, 1989; Meshul et al., 1992). As in humans, these atypical antipsychotics have a low incidence of producing dyskinesias when given chronically to rats (Gao et al., 1998; Kakigi et al., 1995). At the ultrastructural level,

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clozapine produces minimal changes in certain types of striatal synapses which are altered by haloperidol (Meshul et al., 1992). Olanzapine has potent therapeutic actions on the symptoms of schizophrenia (Beasley et al., 1996) and a low potency for producing movement disorders (Tollefson et al., 1997). Olanzapine is similar to clozapine in its chemical structure, receptor affinity profile (Bymaster et al., 1996), and its selective action on mesolimbic vs. nigrostriatal dopamine neurons (Skarsfeldt, 1995). A recent study has shown that rats treated chronically with olanzapine do not develop VCMs (Gao et al., 1998). The goal of the present study was to examine the ultrastructural organization of the striatum of rats chronically treated with olanzapine to test the hypothesis that an antipsychotic without motor side effects would not affect the number of symmetric synapses. This work has been reported in preliminary form (Roberts et al., 1999).

MATERIALS AND METHODS

Drug treatment

Forty-eight adult male Sprague-Dawley (Charles River, Wilmington, MA) albino rats, initially weighing 200 g, were organized into three drug treatment groups: 1) olanzapine (2.0 mg/kg/day, n = 12); 2) haloperidol (1.5 mg/kg/day, n = 26); and 3) water as the control (n = 10). Prior to drug treatment, animals were allowed 1 week to accommodate to the laboratory conditions. Haloperidol-treated rats received a solution of 2.5 mg haloperidol / 100 ml water to drink; olanzapine-treated rats received a solution of 3.3 mg olanzapine / 100 ml water to drink. Details of the methods for preparing the olanzapine have been described previously (Gao et al., 1998). The haloperidol serum levels observed in rats after administration of the above dosage (6.8 ± 1.1 ng/ml; Gao et al., 1997) is similar to the levels of plasma in psychotic patients (Van Putten et al., 1985). The same haloperidol dose, method of drug delivery, or length of treatment do not result in significant changes in food and water intake and body weights (Gao et al., 1997). The dose of olanzapine selected for the present study was based on a dose–response study showing that this dose in rats produces a drug plasma level of 9.3 ± 7.4 ng/ml (Gao et al., 1998), which is within the human therapeutic range (Beasley et al., 1996). The substantia nigra was used from these rats in a concurrent study whose design required a subset of the high VCM rats to be withdrawn from drug for a period of time. Thus, to ensure that enough rats would end up in the high VCM group in order to subdivide it, 26 rats were used. Testing several hypotheses on the same group of rats ultimately results in fewer numbers of animals.

Behavior testing

VCMs were assessed as described previously (Gao et al., 1997; Roberts et al., 1995; Tamminga et al., 1990). Each rat was observed in an empty Plexiglass cage (30 × 18 × 25 cm) by a rater blind to the treatment groups; animals were allowed 2 min to accommodate to the cage followed by a 5-min test period. Testing was conducted at baseline before drug treatment and then monthly during the 6-month treatment period. In the last month, animals were tested twice and VCM scores were averaged; if the rat received one low VCM score and one high VCM score, it was tested two more times and the final score was the average of the last four tests. Rats were divided into a low VCM group (<8 VCMs/5min) and a high VCM group (≥8 VCMs/5min). The score of ≥8 VCMs/5 min has been identified by mixture analysis to be the point of dichotomy between low and high VCMs (Hashimoto et al., 1998). VCMs are represented as group means ± standard deviation.

Tissue preparation

After six months of treatment, rats were withdrawn from drug for 3 days and then sacrificed as detailed previously (Roberts et al., 1995). The reason for the 3-day drug withdrawal is to allow for a washout period, which is important for receptor binding studies. Since many studies of neuroleptic-induced VCMs utilize receptor binding, for comparative purposes the 3-day washout was used in the present study. Thus, rats were deeply anesthetized with a mixture of ketamine/xylazine (5 mg ketamine + 1 mg xylazine/100 g body weight) and perfused transcardially with 50 ml of 0.9% saline followed by 500 ml paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 (PB). All animals were treated according to institutional guidelines. Brains were postfixed for 18–72 h in paraformaldehyde in PB (4°C) and coronally sectioned with a vibratome (40 μm thick); six series of sections were collected.

Tissue was processed for electron microscopic analysis using standard techniques. Briefly, the sections were rinsed in PB (3 × 10 min), immersed in 1% osmium tetroxide for 1 h, stained en bloc with 1% uranyl acetate for 2 h, dehydrated in alcohols, infiltrated with resins, embedded flat on slides, and heated at 60°C for 72 h. Blocks of tissue were removed from the anterior half (rostro-caudal axis) and central region (medio-lateral-dorso-ventral axis) of the striatum (Fig. 1B), mounted on resin beam capsules, thin-sectioned, and examined with the electron microscope. This region was selected based so these results could be compared to our previous work showing anatomical changes in haloperidol-treated rats in this location (Roberts et al., 1995).

Striatal volume

Striatal volume was determined to assess whether or not olanzapine affected striatal volume. Every sixth section (240 μm apart), was stained with cresyl violet

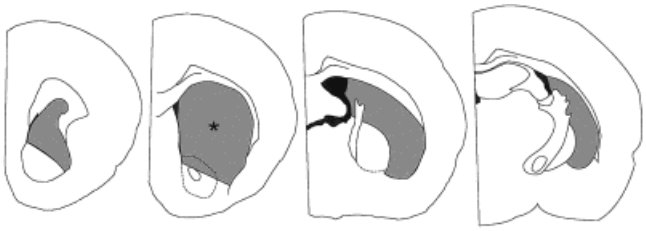

Fig. 1. Schematic illustration showing representative sections from the rostrocaudal extent of the region of the striatum where measurements were taken. The extent of the striatum is large and the shape somewhat irregular. Thus, the striatum was measured within the shaded boundaries. The most anterior section fell within the first 240 μm of the anterior pole of the striatum. In the second section, the asterisk denotes the placement of the sample taken for ultrastructural analysis. The third section is at the level of the globus pallidus. The last section measured was at the level where Ammon's horn first became visible.

and used for volume analyses. The choice of which of the first six sections would be used was determined at random according to the Cavalieri method as described in Ingham et al. (1998). A schematic illustration showing representative sections from the rostrocaudal extent of the striatum is shown in Figure 1. The striatum was measured using similar, but not identical, boundaries as those of Ingham et al. (1998). Thus, the most anterior section fell randomly within the first 240 μm of the anterior pole of the striatum (Fig. 1A), while the most posterior section was measured at the level where Ammon's horn first becomes visible (Fig. 1D). This sampling scheme yielded approximately 16 sections per series per rat. The volume of the striatum was then calculated using Stereologer software (Systems Planning and Analysis, Alexandria, VA). The numbers of rats analyzed per group were: control (n = 5), olanzapine (n = 9), haloperidol low VCM (n = 6), haloperidol high VCM (n = 6), haloperidol combined (n = 12).

EM data collection and analyses

For this analysis, seven olanzapine-treated rats and five water-treated controls were studied; all selected rats had low VCM scores. Striata from three of the five control rats and six of the seven olanzapine-treated rats used for EM were also measured for volume analysis. Animals with the best ultrastructural integrity were selected for analysis. Electron micrographs of the neuropil were taken (@ 10,000×) from serial sections. A montage of six micrographs (average area = 282 ± 45 μm^2 for olanzapine and 278 ± 23μm^2 for controls) was photographed in an average of six sections per animal. The final viewing magnification was 25,000–30,000×.

To determine the density of synapses, the disector stereological technique (Geinisman et al., 1996; Sterio, 1984) was used. Briefly, boxes were drawn in each series of micrographs denoting the neuropil present in every micrograph. Synapses intersecting the left and lower edges of the box were included in the analysis, whereas those intersecting the upper and right edges were not included. In each ribbon of sections, synapses were identified and counted if they disappeared in the look-up section. Thus, sections 1&2 comprised disector 1, sections 2&3 comprised disector 2, etc. (as described in Geinisman et al., 1996). The number of synapses is expressed throughout the text as mean density ± standard deviation / 1 μm^3.

The densities of the following synaptic types were tabulated: all unlabeled synapses combined, axospinous synapses (divided into either symmetric or asymmetric subtypes), axodendritic synapses (divided into either symmetric or asymmetric subtypes), asymmetric synapses (divided into either axospinous or axodendritic subtypes), symmetric synapses (divided into either axospinous or axodendritic subtypes), synapses with perforated postsynaptic densities (perforated synapses). The criteria for identifying a synapse were parallel pre- and postsynaptic membranes, a postsynaptic density and the presence of vesicles near the synapse in the presynaptic element. The raw number of synapses examined per animal was 95.6 ± 21.8 synapses per rat for the controls, and 94.6 ± 21.9 synapses per rat for the olanzapine-treated group; the number of raw synapses per group was not significantly different between groups.

Statistics

Striatal volume and VCM scores were analyzed by a one-way ANOVA followed by a post-hoc Student's independent *t*-test (to compare control, olanzapine, low and high VCM haloperidol groups) or control, olanzapine, and haloperidol groups. To assess drug-induced ultrastructural changes, the data were organized into two groups (control, olanzapine). Unless otherwise mentioned, *P*-values throughout the text refer to the results of post-hoc *t*-tests.

RESULTS

Mean final body weights were similar among the three drug treatment groups: 1) the control group weighed 859 ± 138 g; 2) the olanzapine group weighed 868 ± 157 g; and 3) the haloperidol group weighed 868 ± 61 g. Furthermore, there was no difference between the average weights of the low-VCM haloperidol-treated rats (862 ± 84 g) and the high-VCM haloperidol-treated rats (874 ± 39 g).

Overall group VCM scores are presented in Figure 2. VCM scores differed among the three treatment groups (ANOVA, $P < 0.05$). The VCM scores of the olanzapine group did not differ from those of the control or low-VCM haloperidol groups. The olanzapine group (and controls) had significantly lower VCM scores than those of the haloperidol group as a whole ($P < 0.02$), as well as the subset of haloperidol rats with high VCMs ($P < 0.001$). Some individuals in the olanzapine group (n = 3) had high VCM scores, but neither the propor-

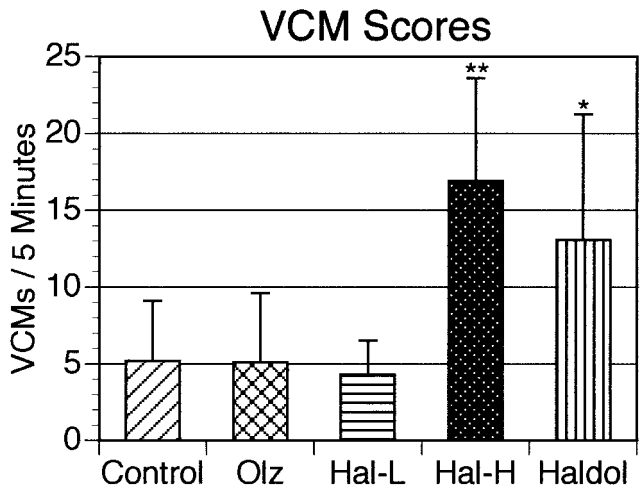

Fig. 2. Histogram illustrating the behavioral scores at study end for control, olanzapine- (Olz), and haloperidol-treated rats (Hal-L = low-VCM rats, Hal-H = high-VCM rats, and Haldol = combined scores for low- and high-VCM rats). Note that the VCM scores of the Olz group did not differ from that of the controls or the haloperidol-treated rats with low VCMs. The VCM scores of the olanzapine-treated group were significantly lower than that of the Hal-H group (**$P < 0.001$) and the haloperidol group as a whole (*$P < 0.02$). Error bars indicate standard deviation.

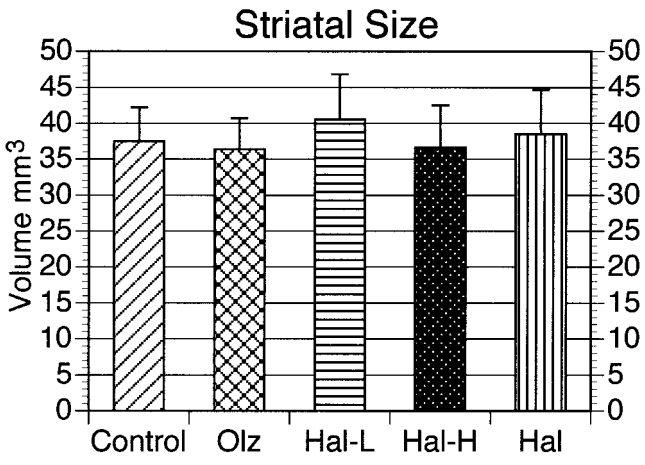

Fig. 3. Histogram showing striatal volumes for control, olanzapine-, and haloperidol (Hal)-treated rats (see abbreviations in Fig. 2). The volume of the striatum, determined from stereological methods, was not statistically different among the groups. Since there were no significant effects on volume, this feature was not included in the analysis of synaptic density. Error bars indicate standard deviation.

tion of cases (n = 3 out of 11), nor the scores (11.9 ± 0.4) differed from those control rats (n = 3 out of 10) that developed spontaneous VCMs (9.6 ± 0.6). Moreover, the high VCMs developed by those rats in the control and olanzapine groups were not as high as typical for the high-VCM haloperidol group (8.5–27.5). In the haloperidol-treated group, eight rats were in the low-VCM group and 18 rats were in the high-VCM group. Nine of the rats with high VCM scores were withdrawn from drug (for 1 month) prior to sacrifice for use in a concurrent study.

There were no between-group differences in striatal volume among the three drug treatment groups (ANOVA, $P > 0.05$). Thus, the volume of the striatum in the olanzapine group did not differ significantly from that of the control or haloperidol-treated groups (Fig. 3). Moreover, striatal volume did not differ between the control group and the haloperidol group — or the control group and either the low- or high-VCM haloperidol groups.

The ultrastructural organization of the striatum is reported here for the control and olanzapine cases, but not the haloperidol cases, as this has been studied previously (Lapidus et al., 1998; Roberts et al., 1995). The ultrastructural integrity of the striatum in the olanzapine-treated rats was within normal limits when compared to that of the control group (Figs. 4, 5). Striatal synaptic organization did not differ significantly in the olanzapine-treated rats in comparison to the controls (Fig. 6). Thus, there were no significant differences in overall combined synaptic density nor in the densities of any of the subgroups that were studied.

DISCUSSION

The results of the present study show that olanzapine given chronically to rats produced VCM scores similar to that of controls and produced no changes in striatal size or alterations in synaptic density. The observation that olanzapine given at clinically relevant doses produces VCM scores similar to controls is consistent with a previous study in rodents examining the behavioral response to this atypical antipsychotic in a dose–response paradigm (Gao et al., 1998) and with clinical data showing very low TD liability (Tollefson et al., 1997). Although it is possible that massive doses of olanzapine may cause both VCMs and/or ultrastructural changes, it is important to note that at clinically relevant doses none of these changes are evident.

Striatal size enlarges by a small degree (5–8%) in schizophrenic individuals and some experimental animals treated with typical neuroleptics (Chakos et al., 1994, 1998), although the cellular compartment responsible for this size change is unknown. In the present study as well as a previous study (Lapidus et al., 1998), no significant changes in striatal volume were observed after chronic treatment with haloperidol, neither in the group as a whole nor in the dyskinetic subset. The functional significance of slight enlargement in striatal size following typical antipsychotics is unknown, but may not be related to TD, as the time course of striatal size changes and the emergence of TD do not match. Nevertheless, in the present study olanzapine did not cause changes in striatal volume.

Overall synaptic density, as well as the density of several subtypes of synapses, were normal in the olanzapine-treated rats. Our results with olanzapine are consistent with those of Meshul et al. (1992), who have shown few ultrastructural effects in rat striatum with

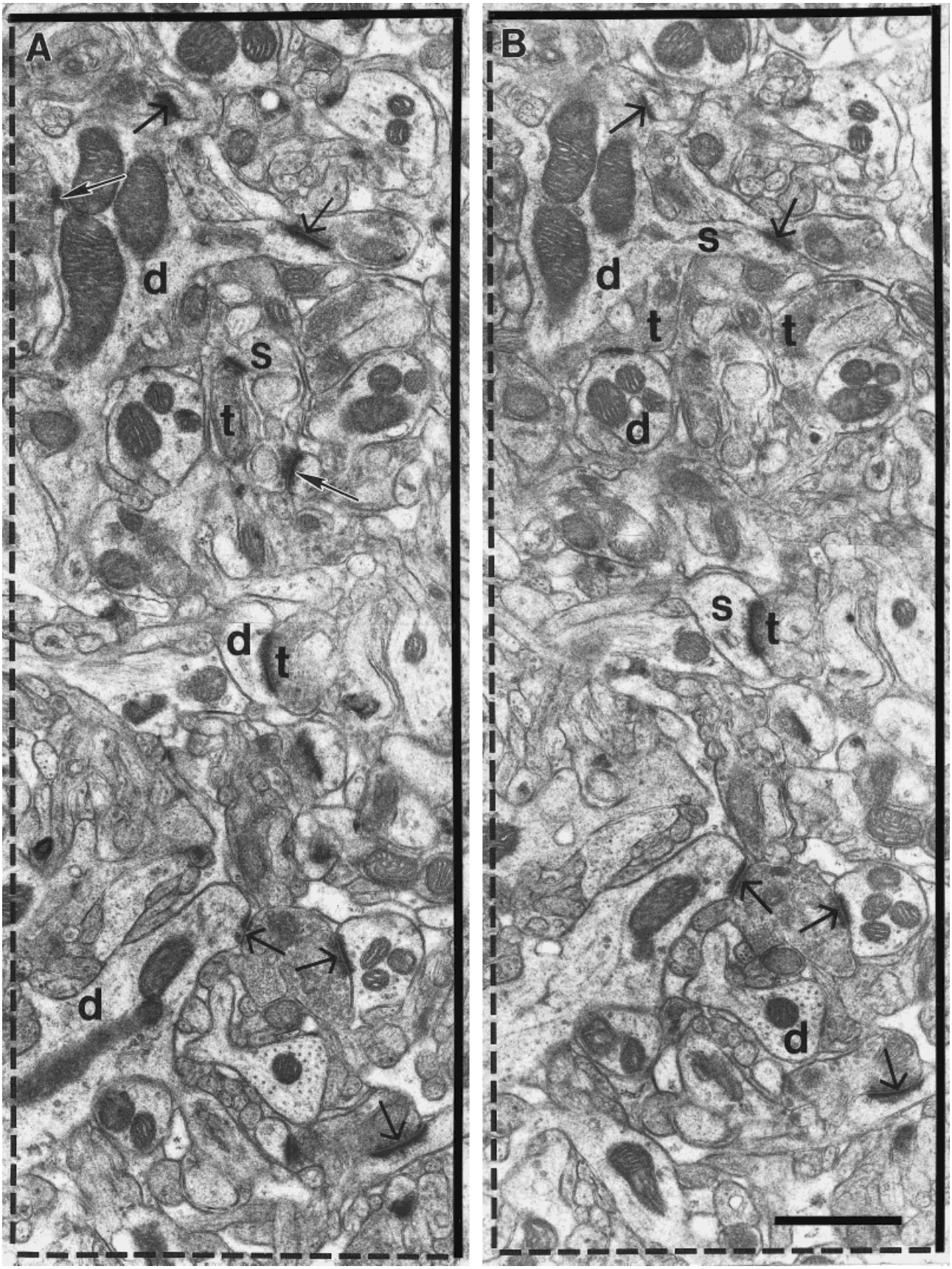

Fig. 4. A sample dissector from a control case. In order to show a larger field of neuropil, the final viewing magnification of these micrographs (22,000X) is smaller than what was used for analysis (25–30,000X). Synapses are counted if they appear in one section, but not the look-up section. Synapses intersecting the left and lower edges (dotted lines) are counted, while synapses intersecting the top and right edges (solid lines) are not counted. Highlighted arrows point to synapses present in section (**A**) but not the look-up section (**B**). Simple arrows point to synapses present in both sections. The area of the counting box is 34.3 μm^2. d, dendrite; s, spine; t, terminal. Scale bar = 1 μm.

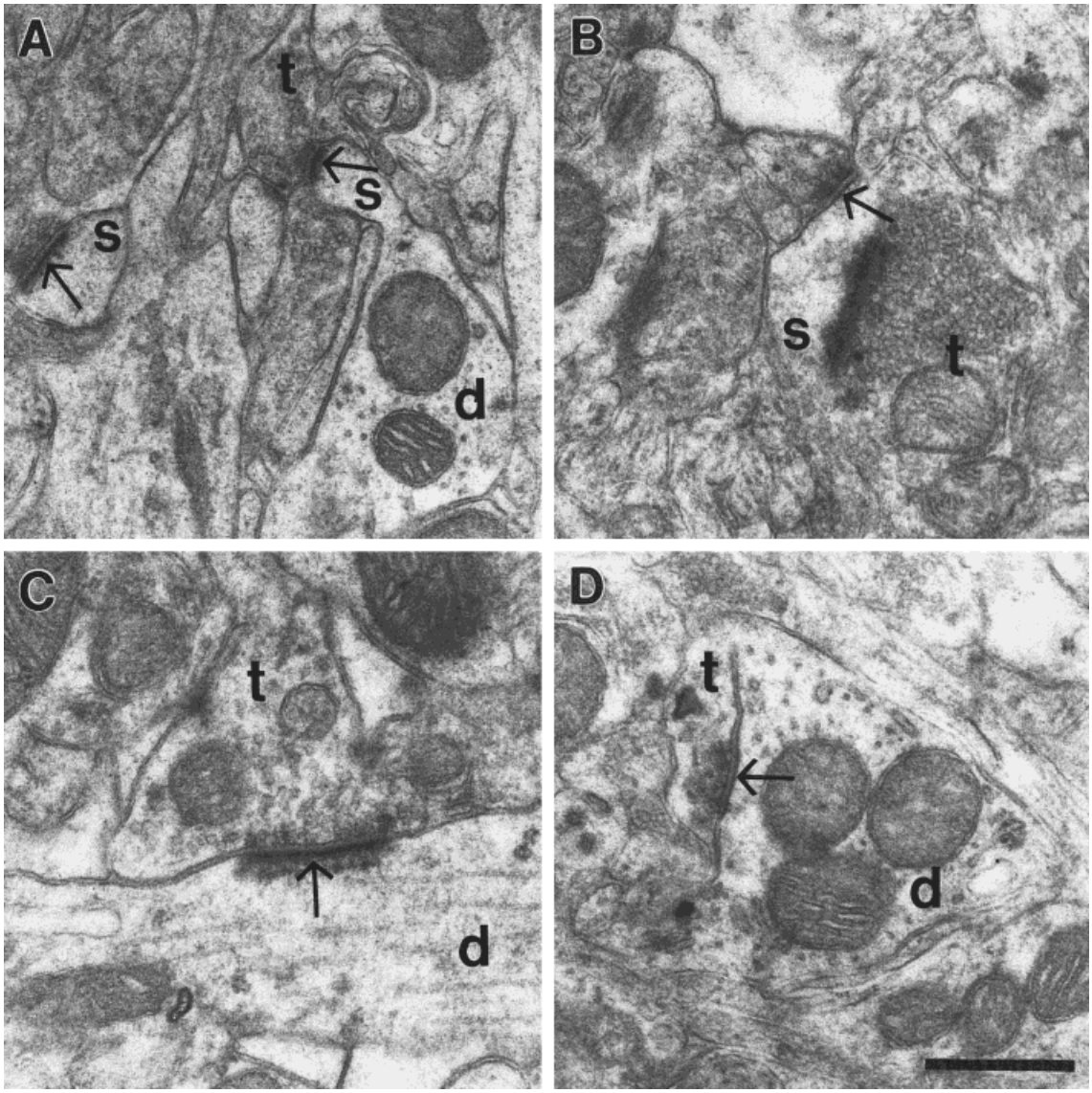

Fig. 5. Examples of different types of synapses (arrows) in an olanzapine-treated animal: (**A**) asymmetric axospinous, (**B**) symmetric axospinous, (**C**) asymmetric axodendritic, (**D**) symmetric axodendritic. d, dendrite; s, spine; t, terminal. Scale bar = 0.5 μm.

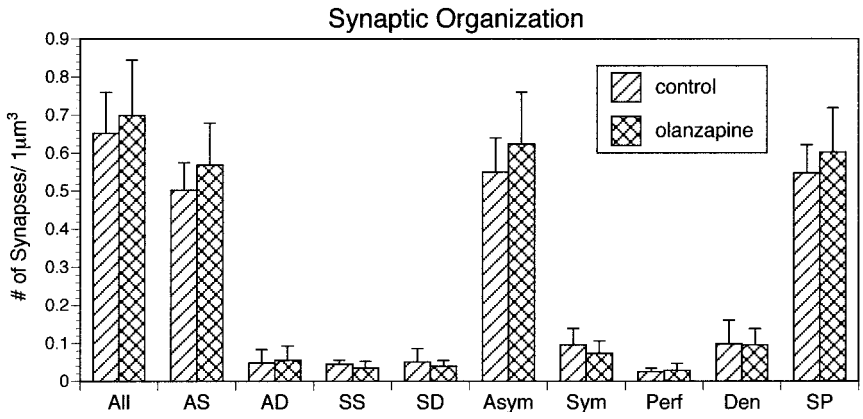

Fig. 6. Histogram showing the synaptic organization of the central region of the striatum in control and olanzapine-treated rats. Synaptic density derived from stereological analysis (no. of synapses / 1 μm^3) is illustrated. No difference was detected between groups in the density of all synapses combined or for the subcategories of synapses shown. All (all synapses combined); AS, asymmetric axospinous; AD, asymmetric axodendritic; SS, symmetric axospinous; SD, symmetric axodendritic; Asym, all asymmetric synapses; Sym, all symmetric synapses; Perf, perforated synapses; Den, all axodendritic synapses; SP, all axospinous synapses. Error bars indicate standard deviation.

another atypical antipsychotic, clozapine. In contrast, our previous studies in haloperidol-treated rats identified a loss of striatal asymmetric synapses, a change which was affected by a similar magnitude in rats with low and high VCMs (Lapidus et al., 1998; Roberts et al., 1995). Since this change was noted in animals with and without dyskinesias, it is apparently a drug effect rather than related to the dyskinetic behavior. Striatal asymmetric synapses are of predominantly cortical or thalamic origin, suggesting a neuroleptic-induced decrease in excitatory neurotransmission (Kemp and Powell, 1971). If the reduction of asymmetric synapses in haloperidol-treated rats is indicative of pathology after such treatment, then the lack of these ultrastructural changes in the olanzapine-treated striatum would suggest a more benign effect of this drug than of haloperidol. If, however, reduction of asymmetric synapses in haloperidol-treated rats is involved with the antipsychotic action of the drug, then the lack of striatal ultrastructural changes in olanzapine-treated rats suggests that the site of action of this drug is elsewhere. In fact, olanzapine, unlike haloperidol, fails to activate nigrostriatal dopamine neurons (Skarsfeldt, 1995) and fails to stimulate c-fos in the striatum (Robertson and Fibiger, 1996), consistent with the latter interpretation.

Our previous data demonstrated that striatal symmetric synapses were decreased in number only in haloperidol-treated rats with high VCMs (Lapidus et al., 1998; Roberts et al., 1995). Symmetric synapses, characteristic of striatal interneurons (DiFiglia, 1987; Ribak et al., 1979; DiFiglia and Aronin, 1984), collaterals of striatal projection neurons (Kitai et al., 1979; Somogyi et al., 1981; Wilson and Groves, 1980), and afferents such as nigrostriatal inputs (Kubota et al., 1987a,b; Pickel et al., 1981) are typically inhibitory in nature. The decrease in density of symmetric synapses was reversible upon drug withdrawal and cessation of VCMs (Roberts et al., 1995). This correlation between a decrease of striatal symmetric synapses and the emergence of oral dyskinesias is consistent with, but does not in itself prove, that the loss of symmetric synapses might cause VCMs. If decreases in the density of symmetric synapses are, in fact, involved in the pathology of VCMs, it is not surprising that such changes are absent after treatment with olanzapine, which has few if any motor side effects (Gao et al., 1998; Tollefson et al., 1997). Thus, the results of the present study support one of the tenets of causality — that the effect (VCMs) and the putative cause (a neuroleptic-induced decrease in striatal symmetric synapses) covary. Experiments to further explore the causal relationship between striatal symmetric synapse loss and neuroleptic-induced oral dyskinesias are under way in our laboratory.

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REFERENCES

Beasley CM Jr, Sanger T, Satterlee W, Tollefson G, Tran P, Hamilton S. 1996. Olanzapine versus placebo: results of a double-blind, fixed-dose olanzapine trial. Psychopharmacology 124:159–167.

Benes FM, Paskevich PA, Davidson J, Domesick VB. 1985a. The effects of haloperidol on synaptic patterns in the rat striatum. Brain Res 329:265–274.

Benes FM, Paskevich PA, Davidson J, Domesick VB. 1985b. Synaptic rearrangements in medial prefrontal cortex of haloperidol-treated rats. Brain Res 348:15–20.

Burt DR, Creese I, Snyder SH. 1977. Antipsychotic drugs: chronic treatment elevates dopamine receptor binding in brain. Science 196:326–328.

Bymaster FP, Calligaro DO, Falcone JF, Marsh RD, Moore NA, Tye NC, Seeman P, Wong DT. 1996. Radioreceptor binding profile of the atypical antipsychotic olanzapine. Neuropsychopharmacology 14: 87–96.

Chakos MH, Lieberman JA, Bilder RM, Borenstein M, Lerner G, Bogerts B, Wu H, Kinon B, Ashtari M. 1994. Increase in caudate nuclei volumes of first-episode schizophrenic patients taking antipsychotic drugs. Am J Psychiatry 151:1430–1436.

Chakos M, Shirakawa O, Lieberman J, Lee H, Bilder R, Tamminga C. 1998. Striatal enlargement in rats chronically treated with neuroleptic. Biol Psychiatry 44:675–684.

Clow A, Theodorou A, Jenner P, Marsden CD. 1980. Changes in cerebral dopamine function induced by a year's administration of trifluoperazine or thioridazine and their subsequent withdrawal. Adv Biochem Psychopharmacol 24:335–340.

DiFiglia M. 1987. Synaptic organization of cholinergic neurons in the monkey neostriatum. J Comp Neurol 255:245–258.

DiFiglia M, Aronin N. 1984. Quantitative electron microscopic study of immunoreactive somatostatin axons in the rat neostriatum. Neurosci Lett 50:325–331.

Egan MF, Hurd YL, Hyde TM, Weinberger DR, Wyatt RJ, Klein JE. 1994. Alterations in mRNA levels of D2 receptors and neuropeptides in striatonigral and striatopallidal neurons of rats with neuroleptic-induced dyskinesias. Synapse 18:178–189.

Gao X-M, Hashimoto T, Cooper TB, Tamminga CA. 1997. The dose-response characteristics of rat oral dyskinesias with chronic haloperidol or clozapine administration. J Neural Transm 104:97–104.

Gao X-M, Sakai K, Tamminga CA. 1998. Chronic olanzapine or sertindole treatment results in reduced oral chewing movements in rats compared to haloperidol. Neuropsychopharmacology 19:428–433.

Geinisman Y, Gundersen HJG, Van Der Zee E, West MJ. 1996. Unbiased stereological estimation of the total number of synapses in a brain region. J Neurocytol 25:805–819.

Gunne LM, Haggstrom J-E. 1983. Reduction of nigral glutamic acid decarboxylase in rats with neuroleptic-induced oral dyskinesia. Psychopharmacology 81:191–194.

Hashimoto T, Ross DE, Gao X-M, Tamminga CA. 1998. Mixture in the distribution of haloperidol-induced oral dyskinesias in the rat supports an animal model of tardive dyskinesia. Psychopharmacology 137:107–112.

Ingham CA, Hood SH, Taggart P, Arbuthnott GW. 1998. Plasticity of synapses in the rat neostriatum after unilateral lesion of the nigrostriatal dopaminergic pathway. J Neurosci 18:4732–4743.

Iverson SD, Howells RB, Hughes RF. 1980. Behavioral consequences of long-term treatment with neuroleptic drugs. Adv Biochem Psychopharmacol 24:305–313.

Kakigi T, Gao X-M, Tamminga CA. 1995. Drug-induced oral dyskinesias in rats after traditional and new neuroleptics. J Neural Transm 101:41–49.

Kelley JJ, Gao X-M, Tamminga CA, Roberts RC. 1997. The effect of chronic haloperidol treatment on dendritic spines in the rat striatum. Exp Neurol 146:471–478.

Kemp JM, Powell TPS. 1971. The termination of fibres from the cerebral cortex and thalamus upon dendritic spines in the caudate nucleus: a study with the Golgi method. Philos Trans R Soc Lond (Biol) 262:429–439.

Kitai ST, Preston RJ, Bishop GA, Kocsis JD. 1979. Striatal projection neurons: morphological and electrophysiological studies. In: Poirier LJ, Sourkes TL, Bedard PJ, editors. Advances in neurology. New York: Raven Press. p 45–51.

Kubota Y, Inagaki S, Kito S, Wu J-Y. 1987a. Dopaminergic axons directly make synapses with GABA neurons in the rat neostriatum. Brain Res 406:147–156.

Kubota Y, Ingaki S, Shimada S, Kito S, Eckenstein F, Tohyama M. 1987b. Neostriatal cholinergic neurons receive direct synaptic inputs from dopaminergic axons. Brain Res 413:179–184.

Lapidus B, Kung L, Glass D, Roberts RC. 1998. Ultrastructural correlates of haloperidol-induced oral dyskinesias in rats: an unbiased stereology study of unlabeled and enkephalin labeled striatal terminals. Soc Neurosci Abstr 24:661.

Meshul CK, Casey DE. 1989. Regional, reversible ultrastructural changes in rat brain with chronic neuroleptic treatment. Brain Res 489:338–346.

Meshul CK, Tan S-E. 1994. Haloperidol-induced morphological alterations are associated with changes in calcium/calmodulin kinase II activity and glutamate immunoreactivity. Synapse 18:205–217.

Meshul CK, Janowsky A, Casey DE, Stallbaumer RK, Taylor B. 1992. Effect of haloperidol and clozapine on the density of "perforated" synapses in caudate, nucleus accumbens, and medial prefrontal cortex. Psychopharmacology (Berl) 106:45–52.

Pickel VM, Beckley SC, Joh TH, Reis DJ. 1981. Ultrastructural immunocytochemical localization of tyrosine hydroxylase in the neostriatum. Brain Res 225:373–385.

Ribak CE, Vaughn JE, Roberts E. 1979. The GABA neurons and their axon terminals in rat corpus striatum as demonstrated by GAD immunocytochemistry. J Comp Neurol 187:261–284.

Roberts RC, Force MM. 1999. The synaptic organization of the striatum in rats chronically treated with olanzapine. Soc Neurosci Abstr 25:377.

Roberts RC, Gaither LA, Gao X-M, Kashyap SM, Tamminga CA. 1995. Ultrastructural correlates of haloperidol-induced oral dyskinesias in rat striatum. Synapse 20:234–243.

Robertson GS, Fibiger HC. 1996. Effects of olanzapine on regional c-fos expression in rat forebrain. Neuropsychopharmacology 14: 105–110.

Skarsfeldt T. 1995. differential effects of repeated administration of novel antipsychotic drugs on the activity of midbrain dopamine neurons in the rat. Eur J Pharmacol 281:289–294.

Somogyi P, Bolam JP, Smith AD. 1981. Monosynaptic cortical input and local axon collaterals of identified striatonigral neurons. A light and electron microscopic study using the Golgi-peroxidase transport-degeneration procedure. J Comp Neurol 195:567–584.

Sterio DC. 1984. The unbiased estimation of number and size of arbitrary particles using the dissector. J Microsc 134:127–136.

Tamminga CA, Dale JM, Goodman L, Kaneda H, Kaneda N. 1990. Neuroleptic-induced vacuous chewing movements as an animal model of tardive dyskinesia: a study in three rat strains. Psychopharmacology 102:474–478.

Tollefson GD, Beasley CM Jr, Tamura RN, Tran PV, Potvin JH. 1997. Blind, controlled, long-term study of the comparative incidence of treatment-emergent tardive dyskinesia with olanzapine or haloperidol. Am J Psychiatry 154:1248–1254.

Uranova NA, Orlovskaya DD, Apel K, Klintsova AJ, Haselhorst U, Schenk H. 1991. Morphometric study of synaptic patterns in the rat caudate nucleus and hippocampus under haloperidol treatment. Synapse 7:253–259.

Van Putten T, Marder SR, May PR, Polan RE, O'Brien RP. 1985. Plasma levels of haloperidol and clinical response. Psychopharmacol Bull 21:69–72.

Waddington JL. 1990. Spontaneous orofacial movements induced in rodents by very long-term neuroleptic drug administration: phenomenology, pathophysiology and putative relationship to tardive dyskinesia. Psychopharmacology 101:431–447.

Wilson CJ, Groves PM. 1980. Fine structure and synaptic connections of the common spiny neuron of the rat neostriatum: a study employing intracellular injection of horseradish peroxidase. J Comp Neurol 194:599–615.