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ORIGINAL INVESTIGATION

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Oral dyskinesias and morphological changes in rat striatum during long-term haloperidol administration

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Abstract *Rationale*: Neuroleptic-induced oral dyskinesias in rats, a putative analogue to human tardive dyskinesia, may be due to increased glutamate release within the striatum. This may lead to excitotoxic degeneration and, as a consequence, persistent motor side effects. *Objectives*: To investigate whether alterations in glutamatergic synapses within the striatum are associated with the development of neuroleptic-induced oral dyskinesia. *Methods*: Haloperidol was administered for 20 weeks, and rats with high and low levels of vacuous chewing movements (VCM) were analyzed for morphological changes with electron microscopy at three time points. *Results*: At week 8, the high VCM rats had a larger nerve terminal area and lower density of nerve terminal glutamate immunoreactivity than the other groups. After 18 weeks of treatment, the nerve terminal area was increased relative to controls in both the high and low VCM groups. After discontinuation of treatment, there were no significant morphological differences between the groups, but the level of VCM was still significantly increased in the high VCM group. *Conclusions*: These results show that striatal glutamatergic transmission is affected during haloperidol treatment and the nerve terminal area and the density of nerve terminal glutamate immunoreactivity are important in determining the VCM response to haloperidol treatment. This indicates that increased glutamatergic synaptic activity in the striatum contributes to the development of human tardive dyskinesia.

Keywords Neuroleptic · Vacuous chewing movement · Glutamate synapse · Nerve terminal area · Glutamate immunolabeling · Tardive dyskinesia

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Introduction

Neuroleptic drugs are used in the treatment of severe mental disorders, such as schizophrenia. However, their long-term use may be limited by unwanted motor disorders, among which tardive dyskinesia (TD) is the most severe. TD is characterized by involuntary movements of the mouth and face, but other parts of the body may also be affected. These movements appear after months of long-term treatment in 20–30% of neuroleptic-treated patients (Kane 1995), and it may persist after discontinuation of treatment. Due to its severity, high incidence, and potential irreversibility, TD is regarded as a major clinical and ethics issue in psychiatry (American Psychiatric Association 1992). The pathophysiological basis of TD remains unclear. In humans, some groups have reported magnetic resonance imaging (MRI) abnormalities (Mion et al. 1991) and histopathological alterations (Miller and Chouinard 1993) in the striatum of TD patients. In rodent models, others have found striatal histopathology (Pakkenberg et al. 1973; Nielsen and Lyon 1978; Jeste et al. 1992) and ultrastructural alterations within the striatum (Benes et al. 1983; Meshul and Casey 1989; Uranova et al. 1991; Kerns et al. 1992; Roberts et al. 1995) associated with antipsychotic drug administration.

It has been proposed that TD may be a result of excitotoxic neurodegeneration in the striatum caused by a

neuroleptic-induced increase in striatal glutamate release (McGeer and McGeer 1976; De Keyser 1991). This is supported by several studies that have reported that neuroleptics increase the basal extracellular level of striatal glutamate (Moghaddam and Bunney 1993; See and Chapman 1994; Yamamoto and Cooperman 1994; See and Lynch 1995) and affect the morphology of striatal glutamatergic synapses in rats (Meshul and Tan 1994; Meshul et al. 1994).

In a rat model of TD, a putative TD analogue, vacuous chewing movements (VCMs) are induced by long-term haloperidol treatment. Using this model, we previously showed that the neuroprotective agent GM1 ganglioside and the *N*-methyl-D-aspartate (NMDA)-antagonist memantine inhibited the development of persistent VCM (Andreassen and Jørgensen 1994; Andreassen et al. 1996), while mitochondrial impairment produced an increase in VCM (Andreassen and Jørgensen 1995; Andreassen et al. 1998). Furthermore, after 1 year of haloperidol treatment, we found an increased percentage of striatal synapses containing a perforated postsynaptic density and that VCM was associated with a larger nerve terminal size (Meshul et al. 1996a). These previous studies support the hypothesis that increased glutamatergic activity in the striatum is associated with VCM.

While our previous rodent study showed ultrastructural changes in the striatum as a result of 1 year of haloperidol treatment (Meshul et al. 1996a), the main goal of the present study was to investigate the possible mechanisms contributing to the development of VCM. The morphological changes in striatal glutamatergic terminals during the early and late phases of long-term haloperidol treatment, as well as after drug withdrawal, were correlated with the severity of oral dyskinesias. This approach enabled us to study the dynamic changes in glutamatergic neurotransmission, and the present results suggest that glutamatergic nerve terminal area and immunoreactivity in the early phase of treatment is important in determining the VCM response to haloperidol.

Materials and methods

Animals and drugs

Female Sprague Dawley rats (Mol:SPRD, Møllegaard, DK, *n*=88) weighing 225–275 g at the start of the experiment were used. The rats were housed under standardized conditions (Andreassen and Jørgensen 1994) and were treated according to the guidelines of the Norwegian Committee for Experiments on Animals. Sixty-eight of the animals were injected with haloperidol decanoate (Janssen, BE) at a dose of 38 mg/kg i.m. every fourth week; 20 control rats were injected with an equal volume of the sesame oil vehicle (Nycomed Pharma, NO). The treatment lasted 20 weeks, with the last injection occurring at week 16.

Behavioral observations

At the start of the experiment, prior to the first videotaping, the rats were handled and habituated to the video recording situation. The behavior of the rats was videotaped in the animal room while they were in a clear Perspex cage (25×11×13 cm) placed in an observation chamber that permitted simultaneous videotaping of the rat from all sides. After 1 min of adaptation to the cage, the rats were videotaped for 3 min. A trained observer, unaware of the treatment group, scored the behavior while watching the videotapes. Using a computer with custom-designed software, the observer scored and analyzed the following behavioral categories: VCM (single, purposeless mouth openings in the vertical plane, with or without tongue protrusions), jerking (sudden body jerks), jaw tremor (high frequency fasciculations of the mouth or jaw), immobility, sitting/standing (no movement of the trunk), moving (horizontal movement of the whole body), rearing, and grooming. For the first two behavioral categories, the frequencies were counted. For the other categories, the duration was recorded.

Protocol

Video recording of the behavior was performed at week 4 and week 6, and then every 4 weeks during treatment and during the 25 weeks after drug withdrawal. After 8 weeks of treatment, 15 rats with the highest levels of VCM, 15 rats with the lowest levels of VCM, and 15 control rats were chosen based on the total number of VCMs obtained on the previous video recordings. The rats were ranked based on the VCM score and every third rat in each group (*n*=5 per group) was selected for morphological analysis. A similar procedure was followed after 18 weeks of treatment; the behavioral data obtained at all the previous time points were added, giving a single value per animal, and the rats were ranked based on the number of VCMs. Then, every second rat in each group was selected, giving five rats per group. The rest of the animals were used for morphological analysis 25 weeks after drug withdrawal.

Tissue preparation for electron microscopy

The selected animals were anesthetized with a mixture of pentobarbital (40 mg/kg) and chloral hydrate (180 mg/kg). The chest cavity was then opened and the rats were perfused transcardially with 500 ml 2.5% glutaraldehyde/0.5% paraformaldehyde in 0.1 M Hepes, pH 7.3, containing 0.1% picric acid. The entire brain was then removed and placed in cold (4°C) fixative overnight. The brains were then washed in 0.1 M Hepes buffer several times and placed into the buffer for 2 days. A 1- to 2-mm^3 piece of the dorsolateral caudate nucleus was then dissected from each animal at a point equivalent to bregma +1.7 mm, according to Paxinos and Watson (1986). This area of the caudate has been used in all of our previous studies and receives a large glutamatergic input from the cortex (McGeorge and Faull 1989; Palmer et al. 1989). The tissue was then incubated in 1% osmium tetroxide/1.5% potassium ferricyanide for 1 h at room temperature, washed several times in water, and placed in aqueous 0.5% uranyl acetate for 30 min at room temperature. It was then dehydrated, cleared in propylene oxide, and embedded in Embed 812/Spurrs at 60°C for no more than 24 h.

Immunocytochemistry

Post-embedding immunogold electron microscopy was performed according to the method of Somogyi et al. (1986) and Phend et al. (1992), as modified by Meshul et al. (1994). The preparation of the tissue is similar to that described above. Thin sections (light gold interference color) were cut and placed on 200-mesh nickel-coated grids, which had been previously double coated with a solution from a Coat Quick “G” pen (Kiyota International; Elk Grove, Ill.). The sections were air dried for several hours and then washed for 5 min in TBST 7.6 solution (0.05 M Tris, pH 7.6, with 0.9% NaCl and 0.1% Triton X100). The grids were then transferred to the primary antibody solution and incubated overnight in a moist chamber. The glutamate antibody (non-affinity purified,

rabbit polyclonal; Arnel, Brooklyn, N.Y.), as previously characterized by Hepler et al. (1988), was diluted 1:100,000 in TBST 7.6. Aspartate (1 mM) was added to the glutamate antibody mixture 24 h prior to incubation with the thin-sectioned tissue to prevent any cross reactivity with aspartate within the tissue. The grids were then washed twice for 5 min and once for 30 min in TBST 7.6, then washed for 5 min in TBST, pH 8.2. The grids were incubated for 1.5 h at room temperature in goat anti-rabbit IgG conjugated to 10 nanometer gold (Amersham, Arlington Height, Ill.; diluted 1:25 in TBST 8.2). The grids were washed twice in TBST 7.6, then deionized water, and counterstained with uranyl acetate and lead citrate.

The sections were viewed and photographed on a JEOL 1200 EX TEMSCAN electron microscope. Photographs (10 per animal; n=5 rats for each group) were taken randomly at an initial magnification of ×25,000 (final magnification=×62,500) throughout the neuropil. The number of gold particles per nerve terminal associated with an asymmetrical synaptic contact was counted and the cross sectional area of the nerve terminal determined using Image Pro Plus software (Media Cybernetics, Md.). The density of immunogold labeling was determined by dividing the number of gold particles per nerve terminal area by the square area of the terminal (no. of gold particles/μm^2). Mitochondria immunolabeled following application of the glutamate antibody were excluded from the nerve terminal analysis but served to determine any changes in the metabolic pool within the nerve ending following drug treatment (Meshul et al. 1999). The specificity of the immunolabeling for the glutamate antibody was established by incubating the antibody overnight with 3 mM glutamate (Meshul et al. 1994). This mixture was then applied to the sections as detailed above. There was no immunogold labeling on the tissue. The total numbers of asymmetrical synapses analyzed in the immunogold density measurements were: control 160, high VCM 143, and low VCM 169 for the 8-week study; 159, 151, and 146, respectively, for the 18-week study; and 126, 114, and 126, respectively, for the 25-week off study.

Statistics

Between-group differences in behavior were analyzed with analysis of variance (ANOVA) followed by Fisher's PLSD post-hoc test to compare group means. Due to non-homogeneity of variance between the different groups, the VCM data were log-transformed (X′=log (X+1)) before statistical calculations. Before the analysis of the behavior, data obtained at different time points were added, giving a single value per animal during the following periods: weeks 4–6 and weeks 12–16 during treatment, and weeks 4–12 and weeks 16–25 after drug withdrawal. The morphological data were analyzed using Peritz' parametric F test for multiple comparison of the means (Einot and Gabriel 1975; Meshul et al. 1999). Correlations were performed with Fisher's r to z test.

Results

Behavior

Four weeks after initiating treatment, there was a significant increase in the level of VCM in all animals receiving haloperidol (2.59±0.26, mean±SEM) relative to the control group (0.45±0.21, P<0.0001). The VCM level in all the haloperidol-treated rats was significantly higher at every time point during treatment (P<0.01–0.0001, data not shown). There was a large variation in the VCM response, and two subgroups with either high or low VCM levels were easily identified (Fig. 1). The difference in the VCM response between these groups was fairly constant during the whole observation period, although there was an overall increase during treatment and then a gradual decrease after cessation of treatment (Fig. 1).

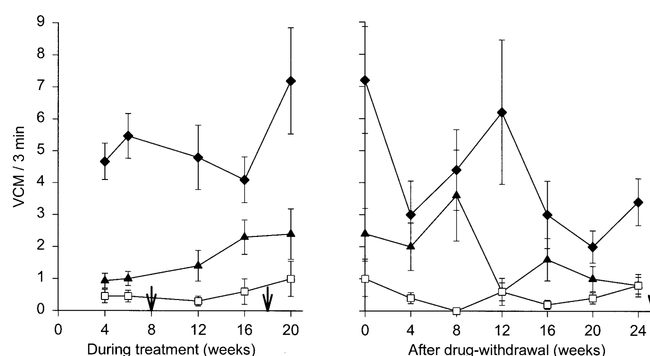

Fig. 1 Number of vacuous chewing movements (VCM)/3 min during long-term haloperidol decanoate treatment, 38 mg/kg i.m. for 4 weeks – the last haloperidol injection was given at week 16. The haloperidol-treated animals were separated into two groups, which demonstrated either a high or low frequency of VCMs [high VCM (▲—▲) and low VCM (◆—◆)]. Control rats (□—□) received vehicle. (Weeks 4–6, n=15 per group; weeks 12–16, n=10 per group; week 20 and after drug withdrawal, n=5 per group). *Arrows* indicate time points for withdrawal of five animals per group for morphological analysis. All data are presented as mean±SEM

During treatment, the high VCM group showed significantly more VCM than the low VCM group (weeks 4–6, P<0.0001; weeks 12–16, P=0.001; and week 20, P=0.05) and the control group (weeks 4–6, P<0.0001, weeks 12–16, P<0.0001, and week 20, P=0.02). The low VCM group showed significantly more VCM than the control group only during weeks 4–6 (P=0.05). Over the whole period of drug administration, the high VCM group showed significantly more VCM than both the low VCM group (P<0.0001) and the control group (P<0.0001), and the low VCM group showed an overall higher level than the control group (P<0.01). After cessation of treatment, the level of VCM in the high VCM group was significantly higher than in the control group both during weeks 4–12 and weeks 16–25 (P=0.02 and P=0.01, respectively), and significantly higher than the low VCM group during weeks 16–25 (P=0.04). The low VCM group was not significantly different from controls.

The other type of oral movements, jaw tremor, followed nearly the same pattern as the VCM development (data not shown). The duration of jaw tremor in the high VCM group was significantly longer than in the control group (weeks 4–6, P=0.008; weeks 12–16, P=0.03) and the low VCM group (weeks 4–6, P=0.015; weeks 12–16, P=0.09). After this time period, the differences in jaw tremor were no longer significant. The level of gross motor activity was reduced by haloperidol only during the early phase of treatment. During weeks 4–6, the high VCM group showed significantly more sitting/standing and less moving/rearing than the low VCM group (P=0.006 and P=0.006, respectively) and the control group (P=0.03 and P=0.008, respectively; data not

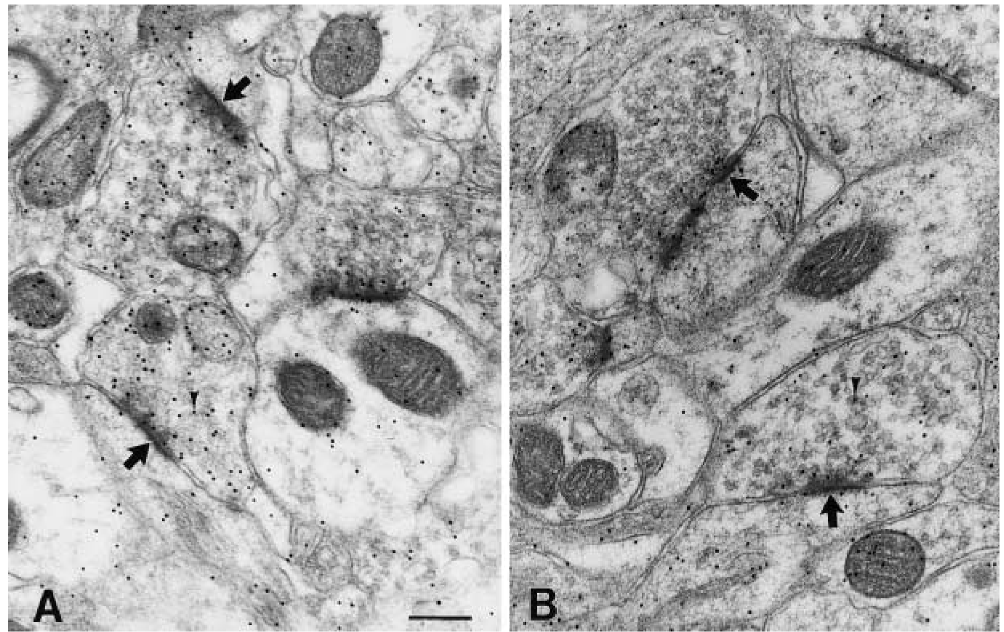

Fig. 2 Electron photomicrograph from the striatum of vehicle- (**A**) and high vacuous chewing movement (VCM; 8-week treatment) (**B**) treated rats illustrating the localization of glutamate using the immunogold method. The glutamate antibody was tagged with a 10-nm gold particle (*arrowheads*), and the highest density is located within the nerve terminal. The terminals are seen making an asymmetrical synaptic contact (*arrows*) with the underlying dendritic spine. Note the decrease in the number of gold particles and the larger terminal size in the high VCM group (**B**) relative to the control group (**A**). *Bar* 0.25 µm

shown). No significant differences were found at later time points.

Glutamate immunocytochemistry

Several examples of nerve terminal glutamate immunolabeling following haloperidol administration have been previously published by our laboratory (Meshul and Tan 1994; Meshul et al. 1994, 1996a, 1996b, 1996c). However, Fig. 2 shows a typical example from the control and high VCM group following 8 weeks of treatment. Numerous gold particles are found concentrated within the nerve terminal relative to the postsynaptic dendritic spine. There is typically a 5:1 ratio in the density of glutamate immunolabeling comparing the nerve terminal with the dendritic spine (Meshul et al. 1994). In addition, glutamate labeling is present in mitochondria within the nerve terminal. The density within this mitochondrial pool can be used as an indication of metabolic changes within the nerve ending and the specificity of the glutamate immunolabeling technique. In all of the treatment groups reported in the current study, there were no changes within the mitochondrial or metabolic pool between any of the groups at any time point studied (data not shown).

Following haloperidol treatment for 8 weeks, there was a significant decrease in the density of nerve terminal glutamate immunolabeling associated with asymmetrical synapses in the high VCM group compared with either the control or low VCM groups (Fig. 3). In the rats selected for morphological studies, there was a significant negative correlation between VCM at 4–6 weeks and the density of glutamate immunolabeling (r=–0.73, P=0.001). In addition, there was a significant increase in the area of the nerve terminal within the high VCM group relative to the control group (Fig. 4).The VCM level at 4–6 weeks was significantly correlated with the nerve terminal area (r=0.63, P=0.01). There was no change in the number of immunogold particles within

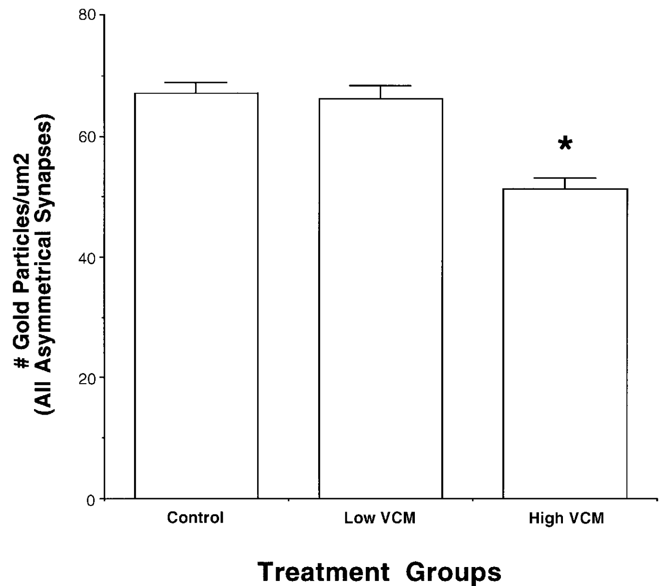

Fig. 3 Quantitative glutamate immunoreactivity within the dorsolateral striatum as demonstrated by the number of gold particles per square micron within the presynaptic terminals of all asymmetrical synapses following treatment with either haloperidol [high or low vacuous chewing movement (VCM) groups] or vehicle (control) for 8 weeks. There was a significant decrease in the density of glutamate immunoreactivity in the high VCM group compared with either the control or low VCM group. There was no significant difference between the control and low VCM group. Values are group mean±SEM. *Significantly different to either the control or low VCM groups using Peritz' F-test for multiple comparisons (P=0.04)

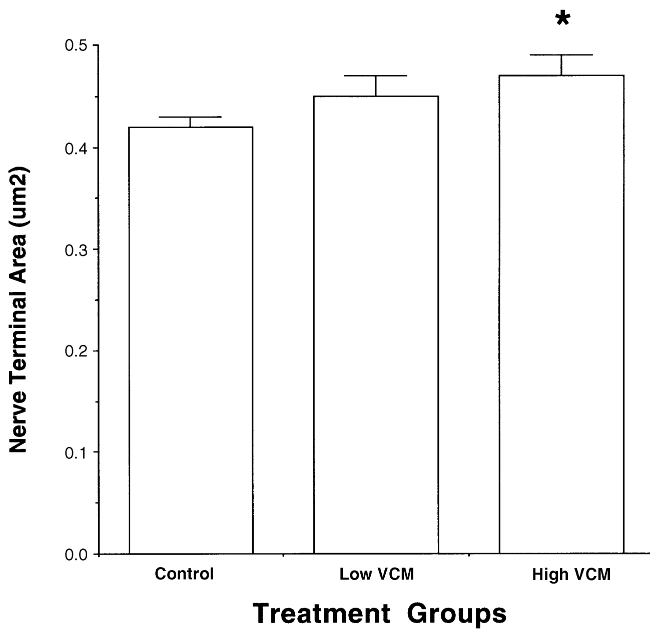

Fig. 4 Nerve terminal area (μm²) of all asymmetrical synapses used in the immunocytochemical analysis described in Fig. 3 (8 week treatment). There was a small but significant increase in the nerve terminal area of the high vacuous chewing movement (VCM) group compared with the control group (P=0.04). There was no significant difference between the low VCM and control groups. Values are group mean±SEM

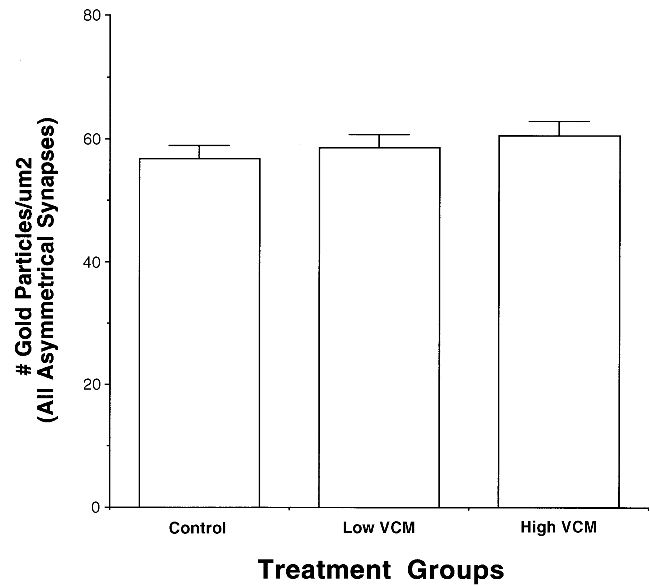

Fig. 5 Quantitative glutamate immunoreactivity within the dorsolateral striatum as demonstrated by the number of gold particles per square micron within the presynaptic terminals of all asymmetrical synapses following treatment with either haloperidol [high or low vacuous chewing movement (VCM) groups] or vehicle (control) for 18 weeks. There were no significant differences between any of the groups. Values are group mean±SEM

the nerve terminals between any of the treatment groups (data not shown).

When the animals were treated with haloperidol for 18 weeks, there was no change in the density of nerve terminal glutamate immunolabeling between any of the groups (Fig. 5). The VCM response from 12 weeks to 16 weeks was not significantly correlated with the immunogold labeling. There was a significant increase in the area of the nerve terminals of both the low and high VCM groups relative to the control group (Fig. 6). In addition, there was a significant correlation between the VCM level from 12 weeks to 16 weeks and the nerve terminal area at 18 weeks (r=0.63, P=0.01). Although there were no density differences between any of the groups at this 18-week treatment period, there was a significant increase in the number of gold particles within the nerve terminals of the low and high VCM groups relative to the control (data not shown). The increase in the number of particles and terminal area accounts for the lack of density differences seen in Fig. 5. There was no significant correlation between the VCM response and either nerve terminal area or immunogold labeling at 18 weeks. When the animals were taken off the drug for 24 weeks, there were no differences between any of the groups in terms of nerve terminal glutamate density or terminal area (Fig. 7 and Fig. 8).

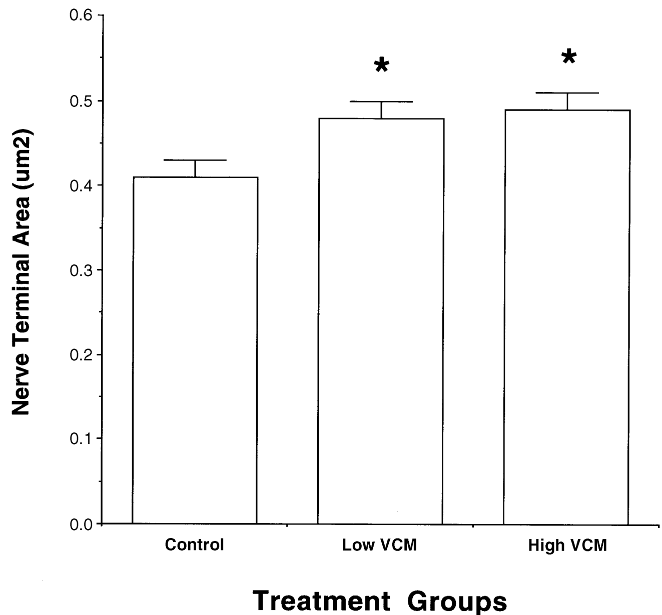

Fig. 6 Nerve terminal area (μm²) of all asymmetrical synapses used in the immunocytochemical analysis described in Fig. 5. There was a small but significant increase in the nerve terminal area for both groups treated for 18 weeks with haloperidol [low and high vacuous chewing movements (VCM)] relative to the control group. There was no significant difference between the low VCM and high VCM groups. Values are group mean±SEM. *Significantly different to the control group using Peritz' F-test for multiple comparisons (P=0.03)

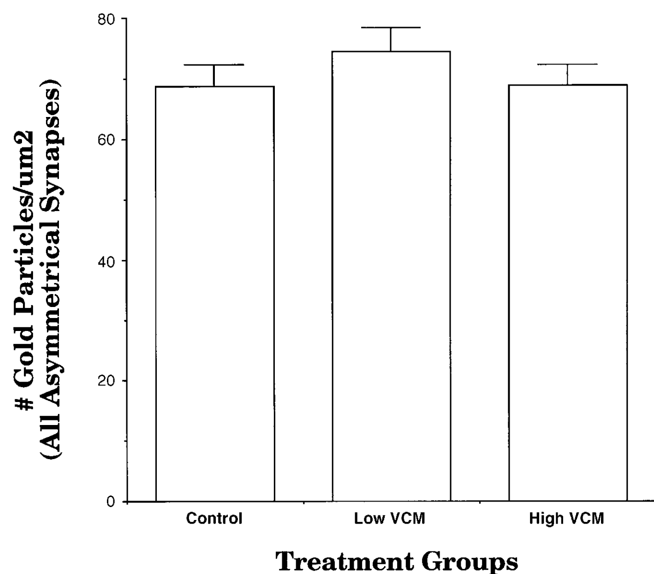

Fig. 7 Quantitative glutamate immunoreactivity within the dorsolateral striatum as demonstrated by the number of gold particles per square micron within the presynaptic terminals of all asymmetrical synapses following treatment with either haloperidol [high or low vacuous chewing movement (VCM) groups] or vehicle (control) for 18 weeks followed by 25 weeks of withdrawal. There were no significant differences between any of the groups. Values are group mean±SEM

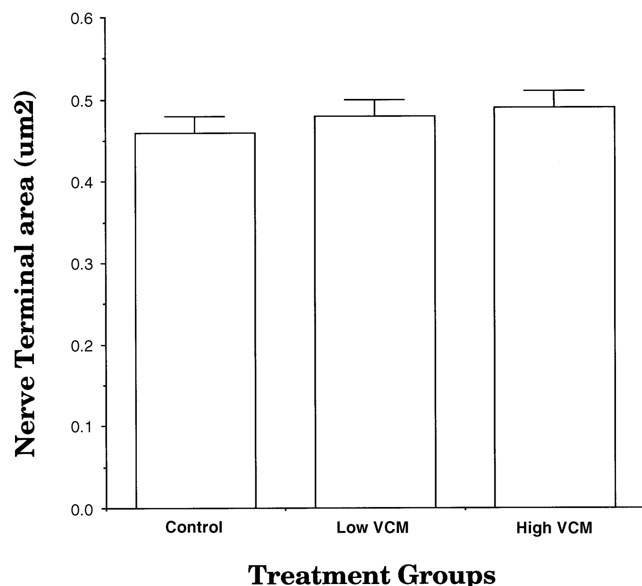

Fig. 8 Nerve terminal area (μm^2) of all asymmetrical synapses used in the immunocytochemical analysis described in Fig. 7. There were no significant differences between any of the groups. Values are group mean±SEM

Discussion

The main finding of the present study is a correlation between neuroleptic-induced oral dyskinesias and altered glutamatergic synapses in the striatum during the development of the motor side effects. In the early phase of treatment, the group of rats with severe oral dyskinesias (high VCM) has significantly larger striatal nerve terminals associated with asymmetrical synaptic contacts and a lower density of striatal nerve terminal glutamate immunoreactivity relative to either the control group or rats that do not develop many dyskinesias (low VCM). After 18 weeks of treatment, there was a significant increase in the nerve terminal area in both groups treated with haloperidol, but there was still a significant correlation to the level of VCM in individual rats. After discontinuation of treatment, the level of oral dyskinesias persisted in the high VCM subgroup, while there were no longer any morphological differences between the groups.

An increase in VCM during long-term neuroleptic treatment has been shown in a number of studies (Clow et al. 1979; Waddington et al. 1983; Gunne et al. 1986; Andreassen and Jørgensen 1994; Egan et al. 1994), including those in which subgroups of rats were shown to be more likely to develop VCM (Tamminga et al. 1990; Andreassen and Jørgensen 1994; Egan et al. 1994; Shirakawa and Tamminga 1994). It has been suggested that subgroups of this type are analogous to humans that develop TD (Egan et al. 1994; Shirakawa and Tamminga 1994; Hashimoto et al. 1998). Benes et al. (1985) first demonstrated that neuroleptics alter the striatal morphology in rats, including increases in the size of neurons and terminals. We and others later reported an increase in the mean percentage of asymmetric synapses with perforated postsynaptic synapses (Meshul and Casey 1989; See et al. 1992; Meshul et al. 1994), and a decrease in the density of asymmetric synapses (Roberts et al. 1995; Meshul et al. 1996a) and dendritic spines (Kelley et al. 1997).

Haloperidol has also been shown to alter the density of glutamate immunolabeling in the striatum (Meshul and Tan 1994; Meshul et al. 1996a, 1996b, 1996c), which is in accordance with microdialysis studies in which neuroleptics were found to increase the extracellular level of striatal glutamate (Moghaddam and Bunney 1993; See and Chapman 1994; Yamamoto and Cooperman 1994; See and Lynch 1995) and to increase the activity of the synaptic protein, calcium/calmodulin kinase II (Meshul and Tan 1994). These changes, however, were not related to the behavioral response. In our previous study (Meshul et al. 1996a), we studied the result of 1 year of haloperidol treatment and found that the VCM level was correlated to morphological alterations. Changes in immunogold labeling for glutamate as well as the nerve terminal area indicated a differentiation between the high and low VCM groups. In the present study, we focused on striatal glutamatergic synapses during the development of VCM. Together, these studies indicate that both decreased glutamate immunolabeling and increased terminal size are critical for the development of VCM, while only increased terminal size seems to be related to chronically high VCM levels during long-term treatment.

Previous work on the striatal effects following a lesion of the nigrostriatal pathway, where glutamate in vivo mi-

crodialysis and immunolabeling were combined, showed that a decreased density of nerve terminal glutamate immunolabeling correlated with an increase in the extracellular level of striatal glutamate (Meshul et al. 1999). This change in extracellular glutamate is commonly interpreted as an indication of an increase in nerve terminal glutamate release. In addition, we have reported that activation of the thalamo-cortico-striatal pathway resulted in an increase in striatal extracellular glutamate, as measured by means of in vivo microdialysis (Meshul et al. 1996b). Therefore, the current decrease in glutamate immunolabelling in the high VCM group in the early phase of treatment (Fig. 3) suggests the possibility of an increase in the release of this neurotransmitter. Another explanation could be that the glutamate re-uptake mechanism is affected. Schneider et al. (1998) showed that neuroleptic treatment decreased the gene expression of the glutamate transporters GLT-1 in the striatum of rats. This may lead to increased extracellular glutamate concentrations and decreased intracellular glutamate levels, which could fit with the observed decrease in immunogold labeling within the terminals in the present study.

The density of glutamate immunolabeling in the high VCM group seems to be decreased mainly because the nerve terminal area was significantly increased, while the absolute number of glutamate immunogold particles was the same as in the other groups. This may suggest that, as the nerve terminal increases in size due to increased synaptic activity (Heuser and Reese 1973), the terminal was able to just maintain its level of glutamate compared with the other two groups. Indeed, an increase in the area of the nerve terminal following increased transmitter release has also been reported using the sympathetic ganglion preparation (Pysh and Wiley 1974). In addition, 1 month following a lesion of the nigrostriatal pathway, in which there is an increase in the extracellular level of striatal glutamate (Meshul et al. 1999), we have recently reported an increase in the size of glutamate-containing nerve terminals (Meshul et al. 2000). Furthermore, we have found that an increase in the extracellular level of striatal glutamate following subchronic nicotine administration is associated with an increase in the area of nerve terminals which are immunolabeled for glutamate (Meshul, unpublished findings). At the end of the treatment period, both the high and low VCM groups showed a significant increase in the number of glutamate immunogold particles per terminal as well as an increase in the terminal area relative to the controls (Fig. 6). The increase in nerve terminal size appears to be consistent with enhanced release of the neurotransmitter (Heuser and Reese 1973), but, at this time point, the terminals were able to increase their level of terminal glutamate by either an increase in synthesis or uptake, resulting in an identical density (i.e., no. of particles/μm^2) of glutamate immunolabeling. After discontinuation of treatment, no alterations in the striatal morphology were observed, possibly reflecting normalized glutamate activity.

The overall results of the current study suggest that haloperidol increases extracellular glutamate levels during treatment in all animals, which is in accordance with previous in vivo microdialysis studies (Moghaddam and Bunney 1993; See and Chapman 1994; Yamamoto and Cooperman 1994; See and Lynch 1995). However, the time point at which the increase in glutamate release is observed appears to be critical for the development of persistent oral dyskinesias. We speculate that the rapid appearance of an increase in extracellular glutamate concentrations could alter the striatal circuitry in a permanent manner, therefore producing the high VCM group. A more gradual and smaller increase in glutamate levels might induce a more reversible change, as found in the low VCM group. A possible mechanism responsible for the persistent changes could be glutamate-induced excitotoxic damage, which is supported by previous work showing that neuroleptic-induced, persistent VCMs in rats are inhibited by the anti-excitotoxic compound, GM1 ganglioside, and the NMDA antagonist, memantine (Andreassen and Jørgensen 1994; Andreassen et al. 1996). The present observation that the glutamatergic alterations are reversible after drug withdrawal while the high VCM levels persist does not rule out the possibility that persistent changes in other striatal functions appear during the period of increased glutamate release. This is supported by persistent high levels of VCM after drug withdrawal observed by several groups (Waddington et al. 1983; Gunne et al. 1986; Egan et al. 1994; Andreassen et al. 1996). Histological evidence for an altered striatal circuitry in rats with pronounced oral dyskinesia comes from two recent studies in which high VCM rats were shown to contain lower numbers of striatal enkephalin and somatostatin neurons than low VCM rats (Andreassen et al. 1999, 2000).

The area of the nerve terminal in the control group and the increase in terminal size following haloperidol has been a consistent finding throughout our ongoing neuroleptic drug studies (Meshul et al. 1992, 1994, 1996a). There have been reports of synaptic alterations (Benes et al. 1983) and swelling of mitochondria (Roberts et al. 1995) during chronic neuroleptic treatment, which could contribute to the increase in terminal area. However, the swelling of mitochondria has never been observed in the current or any of our long-term haloperidol studies (Meshul et al. 1996a). Although some edema/swelling of nerve tissue is apparent in the report of Kerns et al. (1992), it is evident from the photomicrographs presented in Fig. 2 that no such glial swelling was observed in the current study or has ever been shown in any of our previous studies associated with long-term haloperidol treatment (See et al. 1992; Meshul et al. 1996a). Indeed, numerous studies by Benes and associates (Benes et al. 1983, 1985) have also never reported any incidence of haloperidol-induced edema. We believe that such glial or even swelling of mitochondria are due to poor tissue preservation. It is also noteworthy that an increase in striatal volume was observed in rats with pronounced VCM (Chakos et al. 1998) and in humans receiving neuroleptic treatment (Chakos et al. 1994). It might be speculated that the increases in terminal size

could contribute to the increase in striatal volume, which might be related to the motor effects of antipsychotic treatment.

In conclusion, the present results show that striatal glutamatergic transmission is affected during haloperidol treatment and that the nerve terminal area and the density of nerve terminal glutamate immunoreactivity are important in determining the VCM response to haloperidol treatment. This indicates that increased glutamatergic synaptic activity in the striatum contributes to the development of TD.

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