



Short communication

Failure to down regulate NMDA receptors in the striatum and nucleus accumbens associated with neuroleptic-induced dyskinesia

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Abstract

The syndrome of vacuous chewing movements (VCMs) in rats is similar in many respects to tardive dyskinesia (TD) in humans. Both syndromes are characterized by delayed onset of persistent orofacial dyskinesias in a sub-group of subjects chronically treated with neuroleptics. Using the rat model, we examined the role of NMDA receptor-mediated corticostriatal neurotransmission in the expression of VCMs. Rats were treated for 36 weeks with haloperidol decanoate or vehicle and then withdrawn for an additional 28 weeks. Chronic persistent VCMs were induced in one subgroup of treated animals (+VCM), but not in another group (–VCM). Rats from +VCM, –VCM groups and vehicle-treated controls were selected for post mortem studies ($n = 12$ to 14 per group). NMDA receptor levels were assessed using [³H]-MK-801 binding in sections from the mid-striatum and nucleus accumbens. Chronic haloperidol treatment produced a marked reduction of NMDA receptor binding levels throughout the striatum and nucleus accumbens. Post hoc comparisons demonstrated that –VCM rats had lower NMDA receptor binding levels than +VCM and vehicle-treated controls. Ventromedial striatum and nucleus accumbens core were the most affected areas. These findings suggest that down-regulation of striatal NMDA receptor binding levels may protect against the expression of neuroleptic-induced dyskinesia. © 1998 Elsevier Science B.V. All rights reserved.

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Tardive dyskinesia (TD) is a movement disorder seriously affecting the morbidity and mortality of 20 to 40% of all patients medicated with typical neuroleptics on long-term basis. The pathophysiology of TD is unclear and all therapeutic interventions targeting dopaminergic and GABAergic systems are, at best, partially effective [9]. In recent years, a greater understanding of the role of glutamate in regulating striatal functions has emerged. One of the most interesting aspects in relation to TD is the glutamatergic regulation of striatal output. Many studies have revealed that postsynaptic glutamate directly mediates striatal dopamine release via the stimulation of presynaptic *N*-methyl-D-aspartate (NMDA) receptors localized on nigrostriatal nerve terminals [4,11]. In addition, NMDA receptors may indirectly affect dopamine release by modulating the release of other neuro-active molecules such as acetylcholine [10,16], and nitric oxide [17]. On the other

hand, dopamine has been demonstrated to have an inhibitory effect on glutamate release [14] via dopamine D2 receptors located on corticostriatal dendrites [5] and terminals [18].

Preserving the balance of dopamine and glutamate release seems to be a major factor in maintaining normal striatal motor functions [3]. Imbalance of dopaminergic–glutamatergic functions could alter normal striatal activity, possibly resulting in motor abnormalities as in Parkinsonism [6]. Moreover, neuroleptic treatment, which is known to block striatal dopamine receptors, has been shown to alter striatal glutamatergic activity at both structural [12,20] and functional [19,23] levels. Thus, it is intriguing to speculate that neuroleptic-induced alterations in glutamatergic neurotransmission may play a role in TD pathophysiology.

The purpose of the present study is to test the hypothesis of glutamatergic involvement, at the level of striatal NMDA receptor binding, in the expression of neuroleptic-induced dyskinesia using a previously described rat model of TD (for review, see Ref. [7]). We used [³H]-MK-801 to

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assess alterations in NMDA receptor binding levels in the striatum and nucleus accumbens as related to the expression of VCMs in the rat TD model. Receptor autoradiography was performed on mid-striatal sections and receptor densities were compared among +VCM, -VCM, and vehicle-treated controls.

The TD model was created using adult Sprague–Dawley male rats as previously described [7]. The control group ($n = 12$) and the treatment group ($n = 102$) received intramuscular vehicle or haloperidol decanoate (28.5 mg/kg, McNeil Pharmaceuticals) injections, respectively, every 3 weeks for 9 months followed by a 6-month withdrawal period. VCM ratings were done in random order every 3 to 6 weeks (see Fig. 1). At the end of all rating sessions, two selected subsets of the haloperidol-treated animals were assigned to a +VCM group ($n = 14$) and a -VCM group ($n = 14$) according to Egan et al. [7]. A second group of rats weighing 250–300 g received haloperidol (1 mg/kg, Sigma; $n = 6$) or vehicle ($n = 6$) intraperitoneal injections for 21 consecutive days as described previously [8].

Euthanasia, brain removal and sectioning, and haloperidol level assessment were all performed according to Egan et al. [8]. All procedures involving animal use were in strict compliance with the 'NIH Guide for the Care and Use of Laboratory Animals'.

NMDA receptor autoradiography was performed using 30- η M [3 H]-MK-801 (22.5 Ci/mmol, New England Nuclear) according to the method described by Bowery et al. [1]. Non-specific binding was assessed in the presence of 150 μ M of MK-801. Sections were co-exposed with [3 H]

microscales to tritium-sensitive hyperfilm (Amersham) for 3–4 weeks. Striatal receptor density was measured using NIH image analysis system. Striatal sections extended from 1.6 mm to 1 mm anterior to the bregma [15]. Areas of interest were dorsolateral, dorsomedial, ventrolateral, and ventromedial striatum and nucleus accumbens core and shell (Fig. 2a). Each optical density data point was measured bilaterally in two sections per animal, averaged, calibrated and converted to dpm per milligram of wet weight of tissue. Non-specific binding values were subtracted from total binding values in adjacent sections to obtain specific binding data.

Behavioral data were analyzed with repeated measures analysis of variance (rmANOVA) using treatment (haloperidol vs. vehicle) or group (+VCM, -VCM, and control) as the main effect and ratings over time as the repeated measure. Newman–Keuls and Fisher's protected LSD tests were used for post-hoc comparisons between groups and between scores from individual rating sessions, respectively. Calibrated optical density values were analyzed with ANOVA using treatment or group as the between factor and brain region as the within factor. Individual ANOVAs were also done for each brain region. Fisher's protected LSD test was used for specific post hoc comparisons. All data points were described as mean \pm Standard Error of the Mean (S.E.M.) and significant differences were defined at $P < 0.05$.

Statistical analysis of VCM scores showed significant effects of haloperidol treatment ($F = 15.0$, $df = 1$, $P = 0.0002$) and time ($F = 1.9$, $df = 14$, $P = 0.02$). Treatment by time interactions were not significant. Post hoc tests

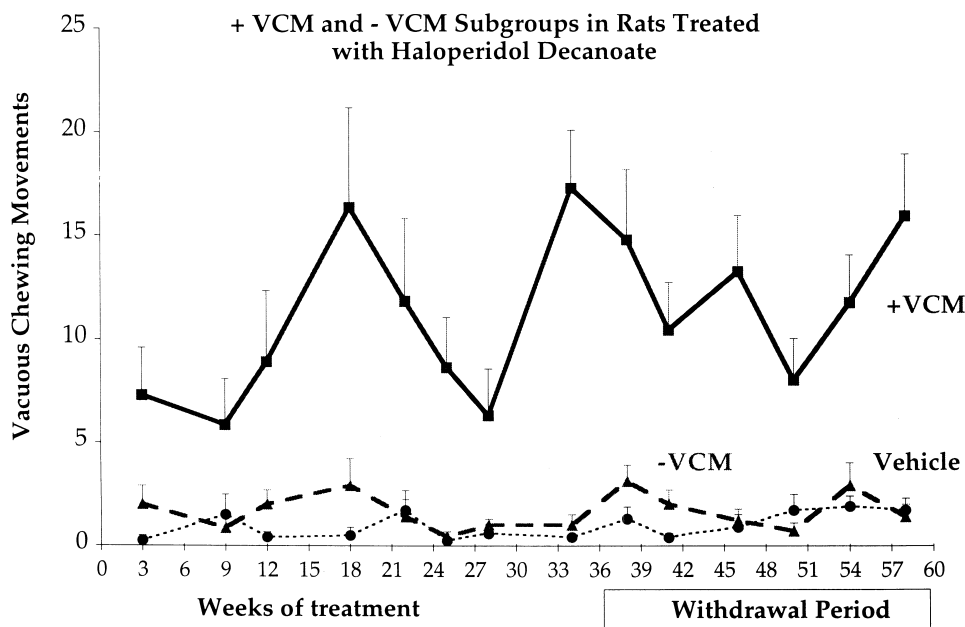


Fig. 1. Effect of chronic haloperidol treatment for 9 months and withdrawal for 6 months on spontaneous jaw movements in rats. Mean VCM ratings for each rating session is reported for haloperidol-treated rats that did (+VCM) or did not (-VCM) develop persistent VCMs as well as for the vehicle-treated control group. Data is expressed as mean \pm S.E.M., *significance at $P < 0.05$ in reference to control.

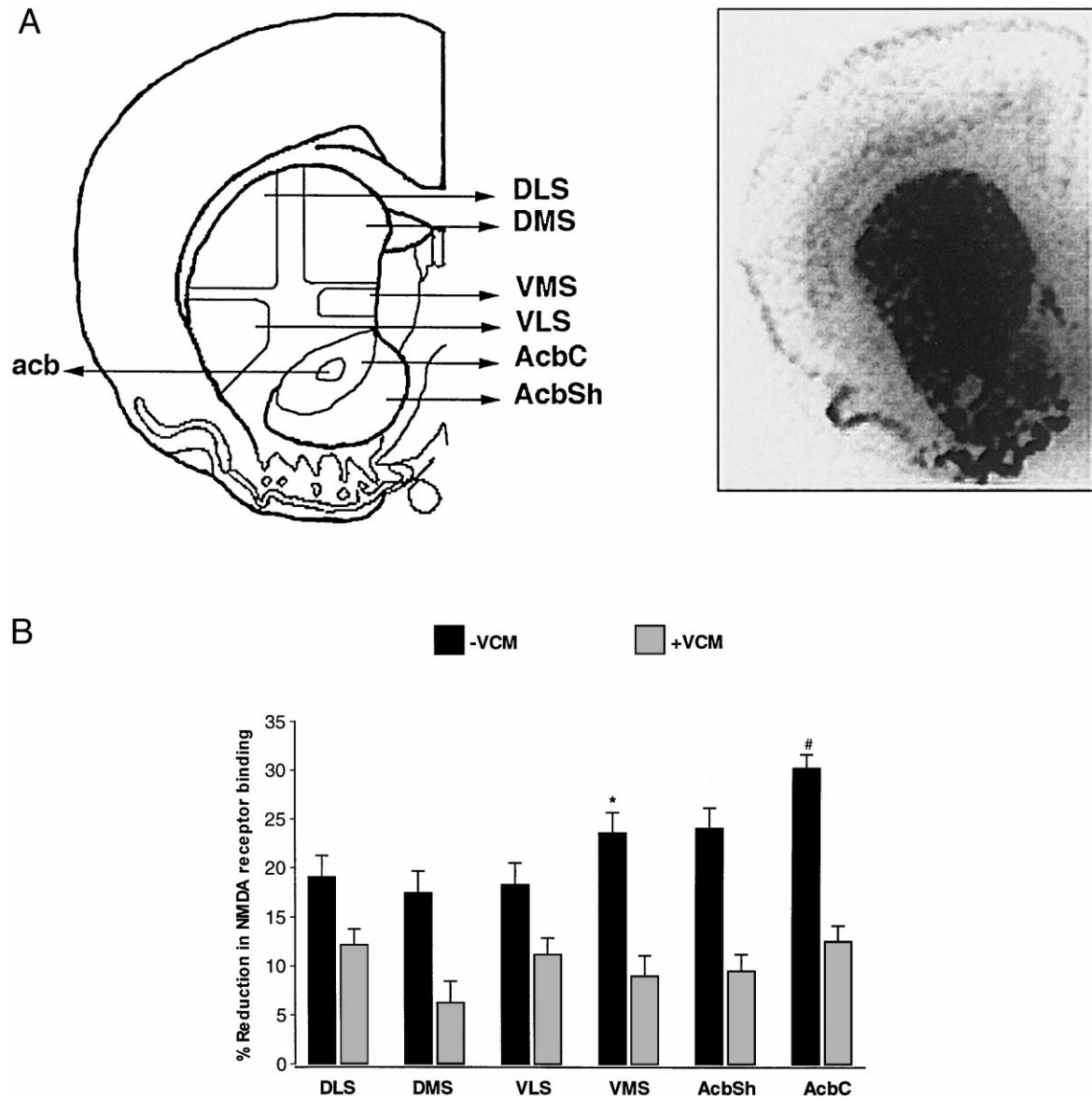


Fig. 2. (a) The regional subdivisions of the striatum and nucleus accumbens used to measure NMDA receptor density (adapted from Ref. [15]). DLS: dorsolateral striatum, DMS: dorsomedial striatum, VLS: ventrolateral striatum, VMS: ventromedial striatum, AcbSh: accumbens shell, and AcbC: accumbens core. (b) Regional alterations in NMDA receptor density levels in the striatum and nucleus accumbens after chronic haloperidol treatment and withdrawal. Striatal divisions are dorsolateral (DLS), dorsomedial (DMS), ventrolateral (VLS), and ventromedial (VMS). Nucleus accumbens is divided into shell (AcbSh) and core (AcbC). Data is expressed as percent change from control in the means of $-VCM$ and $+VCM$ [3H]-MK-801 specific binding \pm S.E.M. *Significance at $P < 0.05$ in reference to control and # indicates significance at $P < 0.05$ in reference to both control and $+VCM$ rats.

showed that VCMs in the haloperidol group were significantly elevated compared to those in the vehicle group starting at the sixth rating session and remained elevated during the entire withdrawal period ($P = 0.056$ – 0.002). $+VCM$ rats showed a marked increase in VCMs compared to control and $-VCM$ groups. The $-VCM$ group did not show any significant increase in VCMs compared to controls (Fig. 1). Analysis showed a significant group effect ($F = 45.7$, $df = 2,37$, $P = 0.0001$), time effect ($F = 2.1$, $df = 13$, $P = 0.01$), and group by time interaction ($F = 1.6$, $df = 26$, $P = 0.03$).

Analysis of NMDA receptor binding levels revealed a significant treatment effect ($F = 4.9$, $df = 1$, $P = 0.038$) was found in rats chronically treated with haloperidol. Mean values for haloperidol-treated animals were lower than the vehicle group. Comparisons of the $+VCM$, $-VCM$, and vehicle groups showed a significant group effect ($F = 7.96$, $df = 2$, $P = 0.003$) and a significant region effect ($F = 8.23$, $df = 5$, $P = 0.0001$). The region by group interaction was not significant. Levels of specific [3H]-MK-801 binding in ventromedial striatum and nucleus accumbens core of $-VCM$ animals were signifi-

cantly lower compared to control ($F = 3.29$, $df = 2$, $P = 0.05$ and $F = 3.64$, $df = 2$, $P = 0.04$, respectively). In post hoc comparison to +VCM rats, receptor binding in –VCM rats was significantly lower in the accumbens core ($P = 0.02$) and showed a trend towards reduction in the ventromedial striatum ($P = 0.07$). No significant changes in NMDA receptor binding density was found in +VCM rats (Fig. 2b) as compared to control. Haloperidol brain levels were not significantly different between +VCM and –VCM rats ($P = 0.5$). Receptor density in mid-striatal sections of rats from the short-term group did not show any significant treatment effect in any of the areas analyzed.

The major finding of the present study is that –VCM rats exhibited a marked reduction in striatal NMDA receptor binding density when compared with both +VCM and control groups (Fig. 2b). Our results are specific to chronic haloperidol treatment and withdrawal since no changes in receptor binding levels were observed after short-term haloperidol exposure, in agreement with a previous finding [13]. Additionally, no significant differences in haloperidol brain levels were observed between +VCM and –VCM rats. Thus, the current data suggests that decreased striatal NMDA receptor binding may offer protection from neuroleptic-induced dyskinesia such as seen in the –VCM group.

The feasibility of altering striatal NMDA receptor binding density by chronic haloperidol treatment is supported by a number of studies. Basal extracellular glutamate concentrations in the caudate and nucleus accumbens were reported to increase after 3 weeks as well as 6 months of haloperidol administration [19,23]. Additionally, both short-term and chronic haloperidol treatment were found to increase the number of glutamatergic perforated synapses within the caudate nucleus while considerably decreasing presynaptic glutamate immuno-gold labelling indicating increased glutamate release. NMDA receptor antagonists were reported to suppress haloperidol-induced increase of perforated synapses (for review, see Ref. [13]). Taken together, these results indicate that neuroleptic drugs act to enhance excitatory striatal neurotransmission, which may be related to their high incidence of extrapyramidal side effects (EPS) [23]. One of the possible mechanisms of neuroleptic-induced glutamatergic overactivation is the disinhibition of corticostriatal neurons as a result of collateral blockade of inhibitory D2 receptors localized postsynaptically on the cortical dendrites [5], and presynaptically on the striatal terminals of these neurons [4].

Therefore, the reduction of striatal NMDA receptor binding levels in –VCM rats could minimize the effects of overactive corticostriatal glutamatergic stimulation on striatal output pathways, particularly those involved in the expression of VCMs. For example, significant elevations of striatal enkephalin and dynorphin gene expression occur in the ventromedial striatum and nucleus accumbens of the +VCM animals only [7]. Both dynorphin and enkephalin

biosynthesis fall under NMDA receptor-mediated glutamatergic regulation [21,22]. Studies have revealed that NMDA receptor blockade inhibits the increase in dynorphin mRNA and peptide expression seen after D1 receptor SKF 38393 activation [2] in addition to reducing the striatal content of enkephalin mRNA [22]. Therefore, reduced NMDA receptor binding levels in –VCM animals may protect against increased levels of dynorphin and enkephalin. This in turn may propagate a relative reduction in the activity of both the striatonigral (direct) and striatopallidal (indirect) output pathways, which may prevent the development of dyskinesias.

In conclusion, our data demonstrate that chronic haloperidol treatment can induce distinct long-term changes in the corticostriatal regulatory pathway, which may play a role in the genesis of the VCM syndrome. Changes in the ventromedial striatum and the core region of the nucleus accumbens are particularly important. To the extent that the VCM syndrome in rats is the analogue of TD in human, similar pathophysiological mechanisms may underlie both movement disorders.

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