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Differential effects of antipsychotics on haloperidol-induced vacuous chewing movements and subcortical gene expression in the rat

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Abstract

The behavioral and neurochemical effects of switching from typical to atypical medications have not been evaluated in the rodent models of tardive dyskinesia. Thus, we treated rats with haloperidol–decanoate for 12 weeks, and assessed the effects of additional treatment with olanzapine, haloperidol, clozapine, or vehicle on vacuous chewing movements and expression of transcripts for dopamine receptors, tyrosine hydroxylase, δ-opioid receptor, prodynorphin, preproenkephalin, glutamic acid decarboxylase-65 (glutamic acid decarboxylase (GAD)-65) and GAD-67 and *N*-methyl-D-aspartate (NMDA) receptor subunits in the striatum and its efferent pathways. Haloperidol–decanoate induced vacuous chewing movements extinguished following an additional 4 weeks of treatment with vehicle, olanzapine or haloperidol, but not clozapine. Post-treatment, vacuous chewing movements in the clozapine group were significantly higher than the vehicle, olanzapine and haloperidol groups. GAD-67 mRNA expression in the globus pallidus was decreased following additional treatment with olanzapine or haloperidol, but not clozapine. Changes in expression of other transcripts were not detected. These findings demonstrate important differences in the effects of typical and atypical antipsychotics on chronic vacuous chewing movements.

Keywords: Haloperidol; Olanzapine; Clozapine; Neuroleptic; Striatum; Oral dyskinesia

1. Introduction

The efficacy of typical antipsychotics for the treatment of psychosis revolutionized the treatment of schizophrenia and other psychotic illnesses. However, typical antipsychotic medications are associated with potentially severe movement disorders such as tardive dyskinesia, a disorder characterized by choreathetotic movements of the mouth, tongue, extremities, and trunk muscles (Sadock and Sadock, 2000). Tardive dyskinesia occurs in approximately 20% of patients who take typical antipsychotics for at least 1 year, and in many cases, symptoms of tardive dyskinesia do not remit after discontinuation of the drug (Glazer, 2000a,b; Jeste et al., 1998). The development of new antipsychotic medications was driven in part by the need to decrease the risk of developing tardive dyskinesia for patients who require chronic antipsychotic treatment. These newer antipsychotics are considered atypical because of their favorable motor side effect profile and efficacy against both negative (deficit) and positive symptoms compared with the older typical antipsychotics (Kinon and Lieberman, 1996; Tarsy et al., 2002). In contrast to the typicals, atypical medications have very low rates of tardive dyskinesia (Caroff et al., 2002; Glazer, 2000a).

Rodent models have been developed to clarify the underlying pathophysiological mechanism of tardive dyskinesia (Clow et al., 1979; Gunne and Growdon, 1982; Gunne et al., 1982; Iversen et al., 1980). Antipsychotic-induced vacuous chewing movements in rats have been proposed as an analogous model of tardive dyskinesia, due to similarities in onset, variability, and duration of abnormal movements (Turrone et al., 2002). Similar to tardive dyskinesia, vacuous chewing movements that result from chronic antipsychotic treatment are masked by increasing doses of typical antipsychotics, may worsen with age, and may persist following discontinuation of treatment (Andreassen and Jorgensen, 2000; Egan et al., 1996; Turrone et al., 2002). Vacuous chewing movements are likely a behavioral manifestation of pharmacological disruption of neurotransmitter

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pathways. The striatum and its efferent pathways are normally modulated by dopaminergic, glutamatergic, and other neurotransmitter systems (Gerfen, 1992). One hypothesis suggests that vacuous chewing movements induced by typical antipsychotics are mediated by dopamine D1 receptors, since dopamine D1 receptor agonists restored vacuous chewing movements in dopamine-depleted rats (Diana et al., 1992; Ohno et al., 1997; Rosengarten and Friedhoff, 1998; Yu et al., 1999). Dopamine D2 receptor supersensitivity has also been proposed, but a dopamine D2 receptor agonist blocked vacuous chewing movements, and intrastriatal injection of dopamine D2 receptor mRNA specific antisense oligonucleotides did not affect apomorphine-induced vacuous chewing movements (Johansson et al., 1987; Rajakumar et al., 1997). An alternative hypothesis suggests that vacuous chewing movements are a result of antipsychotic induced excitotoxicity, based on the observation of attenuated vacuous chewing movements following administration of either memantine, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, or GM1 ganglioside, an inhibitor of the downstream effects of glutamate receptor activation (Andreassen and Jorgensen, 1994; Andreassen et al., 1996; De Keyser, 1991). These competing hypotheses may not be mutually exclusive, since the glutamatergic and dopaminergic systems interface in the striatum and other brain regions implicated in pathophysiology of vacuous chewing movements.

The location of the striatum between the cortex and other subcortical structures, together with reported changes in vacuous chewing movements, striatal morphology and gene expression, suggests a central role for the striatum in rodent models of tardive dyskinesia (Gerfen, 1992; Heimer et al., 1993). The striatum contains two subpopulations of γ-aminobutryic acid (GABA)-ergic projection neurons that give rise to phenotypically distinct circuits that differentially modulate basal ganglia output. The direct (striato-nigral) pathway is comprised of dopamine D1 receptor and dynorphin expressing GABAergic neurons that project to the substantia nigra, pars reticulata (Gerfen, 1992; Heimer et al., 1993). The indirect (striato-pallidal) pathway is comprised of dopamine D2 receptor and enkephalin expressing GABAergic projections to the globus pallidus and ventral pallidum, and pallidal projections directly to the substantia nigra pars reticulata, as well as to the entopeduncular nucleus and substantia nigra, pars reticulata via the subthalamic nucleus (Gerfen, 1992; Heimer et al., 1993). The striatum receives excitatory glutamatergic input from the cortex and hippocampus, and dense innervation from midbrain dopaminergic neurons arising in the motor-associated substantia nigra, pars compacta and limbic associated ventral tegmental area (Gerfen, 1992). Functionally, the direct and indirect pathways act in concert to modulate voluntary movements and other behaviors. Pharmacological disruption of either pathway disturbing the balance of GABAergic tone flowing from the striatum may result in abnormal stereotyped movements (Gerfen, 1992).

Previous studies with the rat vacuous chewing movement model of tardive dyskinesia have been limited to evaluating antipsychotics for induction of vacuous chewing movements. However, in the clinic, many patients with tardive dyskinesia have been switched from the tardive dyskinesia-causing typical to atypical antipsychotics. The goal of such a switch is to stabilize and in some cases improve tardive dyskinesia symptoms (Tamminga et al., 1994). While common in patients, this strategy has not been modeled or studied in rodents in order to gain insight into the mechanism of this atypical-induced stabilization and/or improvement in tardive dyskinesia at the molecular, cellular, and circuitry levels. Thus, the purpose of this study is to evaluate the effects of both typical and atypical antipsychotic medications on vacuous chewing movements previously induced in rats by chronic haloperidol-decanoate treatment. Specifically, we hypothesized that atypical and typical antipsychotics would differentially attenuate chronic, haloperidol-decanoate-induced vacuous chewing movements and differentially regulate gene expression in the striatum and its efferent pathways associated with these behavioral effects.

2. Materials and methods

2.1. Animals and tissue preparation

Seventy-two adult male Sprague–Dawley rats (250 g) were utilized for this study. Twenty-four hours after the last injection, brains from sacrificed animals were removed, frozen in isopentane and stored at −80 °C until sectioned. Each brain was thawed for 30 min to a temperature of −20 °C, and mounted for cryostat sectioning. Ten-micrometer sections were taken in the horizontal plane, mounted on Superfrost Plus microscope slides (Fisher Scientific, Pittsburgh, PA), desiccated, and stored at −80 °C.

2.2. Induction of vacuous chewing movements

Each of the 72 rats received intramuscular injections in the hindlimb of haloperidol-decanoate (15 mg/kg) every 14 days for 12 weeks (induction phase). A total of seven doses of haloperidol-decanoate (15 mg/kg/2 weeks) were administered to each rat, followed by a 4-week washout period after the last injection. This dosing regimen has previously been used by numerous groups for chronic administration of haloperidol and has been shown to induce chronic vacuous chewing movements in rats (Egan et al., 1996; Grimm et al., 1998; Gunne et al., 1982; Oh-e et al., 1991).

2.3. Assessment of vacuous chewing movements

Animals were placed individually in an observation chamber with a clear plastic front and mirrors on three sides and allowed to habituate for 1 min. Animals were monitored

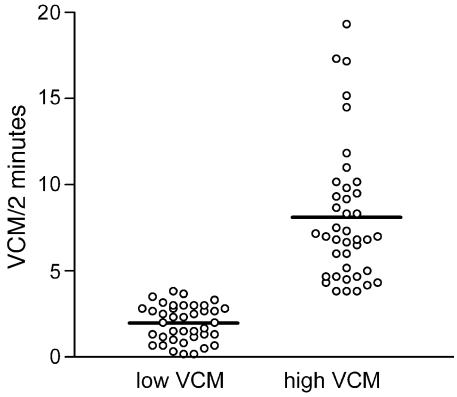

Fig. 1. Vacuous chewing movements (VCMs) in rats following 12 weeks of treatment with haloperidol-decanoate and a 4-week washout period. Animals that received antipsychotic were assigned to "high" or "low" vacuous chewing movement groups. The high vacuous chewing movement group was used for the treatment phase of the study.

for 2 min by two observers blind to the treatment conditions, for purposeless oral chewing movements in the vertical plane that were not associated with grooming, feeding, or exploring the environment. Tongue protrusions and other non-chewing orofacial movements were not counted. The total vacuous chewing movements scored per 2-min test interval by the two observers were averaged for each rat. Vacuous chewing movements were assessed 1, 2, and 3 weeks after the induction phase and weekly during the treatment phase.

2.4. Treatment phase of study

The 40 animals with the highest number of orofacial movements/minute were randomized into four groups of 10 rats receiving daily subcutaneous injections for 4 weeks of olanzapine (10 mg/kg/day), clozapine (20 mg/kg/day), haloperidol (2 mg/kg/day), or vehicle (acidified, 90% dimethyl sulfoxide). This subset of rats was considered the treatment phase of the experiment. During the treatment phase, vacuous chewing movements were measured 24 h after injection of vehicle or active medication.

2.5. In situ hybridization

Subclones for rat dopamine D1, D2, and D3 receptors, δ-opioid receptor, prodynorphin, preproenkephalin, the NMDA receptor subunits NR1 and NR2A-2D, tyrosine hydroxylase, and the 5-HT_{2C} receptor were prepared and characterized as previously described (Healy and Meador-Woodruff, 1999; Ibrahim et al., 2000; Julius et al., 1990; Mansour et al., 1993; Meador-Woodruff et al., 1991, 1992, 1994). Unique areas of

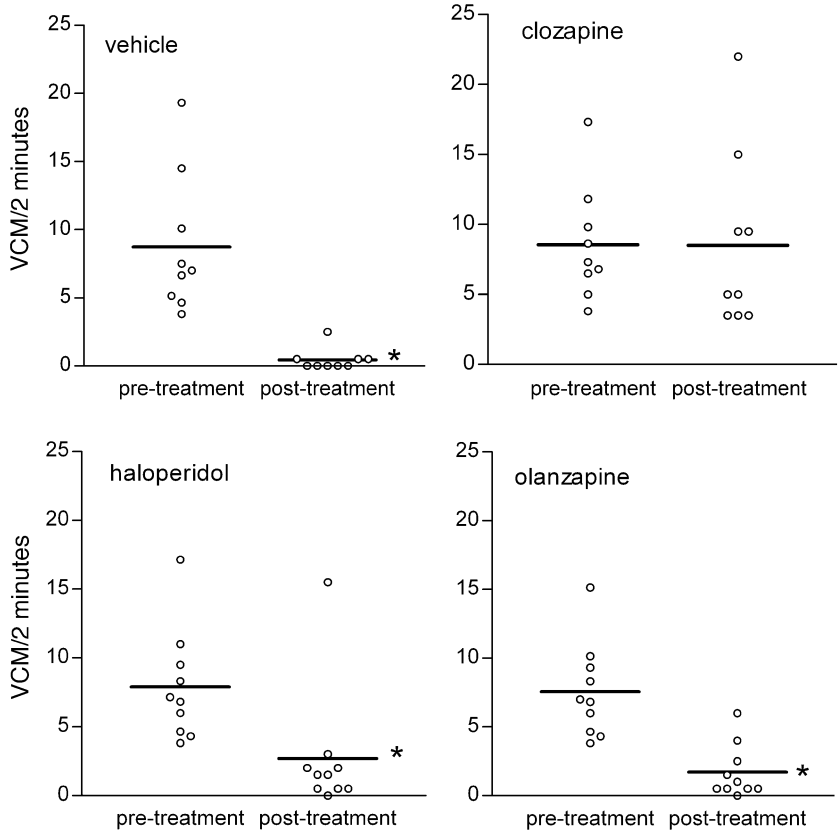

Fig. 2. Vacuous chewing movements (VCMs) in high VCM rats pre- and post-additional treatment with vehicle, haloperidol or olanzapine. We did not detect differences in VCMs between randomized groups of rats prior to the treatment phase. Following the treatment phase, VCMs were significantly decreased in the vehicle, haloperidol, and olanzapine groups, but not in the group treated with clozapine (*$P < 0.05$).

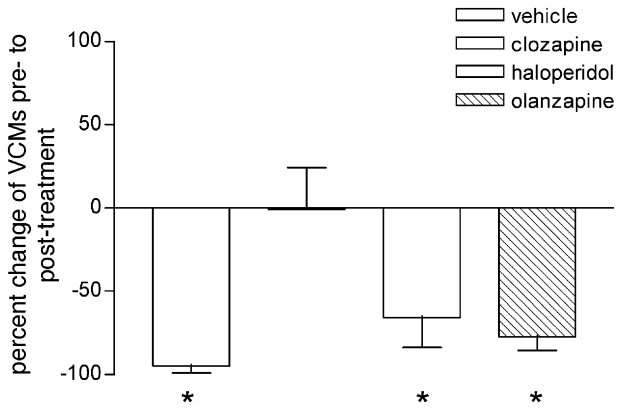

Fig. 3. Percent change in haloperidol-induced vacuous chewing movements (VCMs) following additional treatment with vehicle, clozapine, haloperidol, or olanzapine (*$P < 0.05$).

other genes of interest were targeted for amplification. Primers for polymerase chain reaction amplification were designed for rat glutamic acid decarboxylase (GAD)-65 (genbank accession # M72422, coding region 94-563) and GAD-67 (M76177, 155-645) using Primer 3 (http://www.genome.wi.mit.edu/genome_software/other/primer3.html) and AMPLIFY (http://engels.genetics.wisc.edu/amplify/index.html; Bill Engels, Madison, WI). Following amplification from a rat brain library (Clontech Laboratories, Palo Alto, CA), the DNA was extracted and purified with the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA), subcloned using the Zero Blunt TOPO PCR Cloning Kit (Invitrogen, Carlsbad, CA), and sequenced (Thermo Sequenase Radiolabeled Termination Cycle Sequencing Kit, USB, Cleveland, OH).

For riboprobe synthesis, 10 μl of [^{35}S]uridine triphosphate was dried and 2.0 μl 5× transcription buffer, 1.0 μl 0.1 M dithiothreitol, 1.0 μl each of 10 mM adenosine triphosphate, cytosine triphosphate, and guanidine triphosphate, 2.0 μl linearized plasmid DNA, 0.5 μl RNAse inhibitor, and 1.5 μl SP6, T3 or T7 RNA polymerase were combined and incubated for 2 h at 37 °C. 1.0 μl of DNAse (RNAse-free) was added and the mixture was incubated for 15 min at room temperature. Radiolabeled probe was purified with microspin chromatography columns (Bio-Rad, Hercules, CA). Two slides per animal for each probe for each region of interest were removed from −80 °C storage and placed in 4% (weight/vol) formaldehyde at room temperature for 1 h. The slides were then washed in 2× 300 mM NaCl/30 mM sodium citrate, pH 7.2, three times for 5 min each. Slides were washed in deionized H_2O for 1 min and placed in 0.1 M triethanolamine, pH 8.0/acetic anhydride, 400:1 (vol/vol), on a stir plate, for 10 min. The final wash was in 2× 300 mM NaCl/30 mM sodium citrate, pH 7.2 for 5 min, followed by dehydration

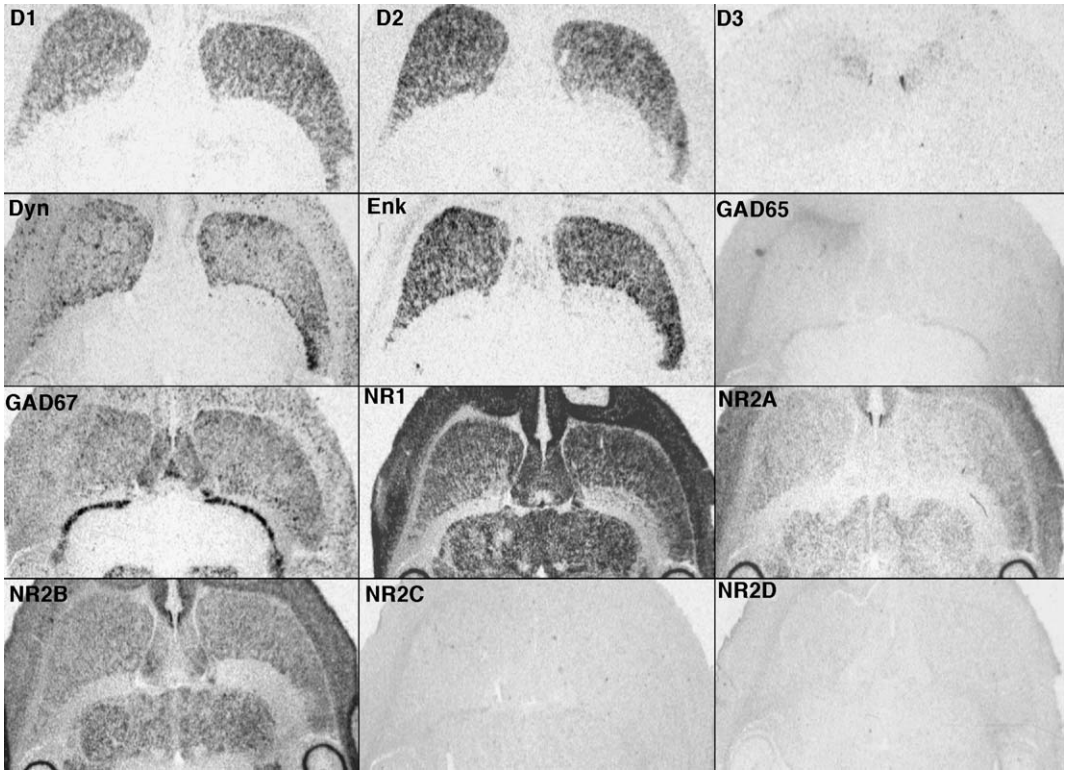

Fig. 4. Gene expression in the striatum shown on horizontal brain sections. Transcripts for dopamine D1, D2 and D3 receptors (D1, D2, and D3), prodynorphin (Dyn), preproenkephalin (Enk), glutamic acid decarboxylase (GAD) 65, GAD67, and the NMDA receptor subunits NR1 and NR2A-D were detected in the caudate-putamen (CPU) by in situ hybridization.

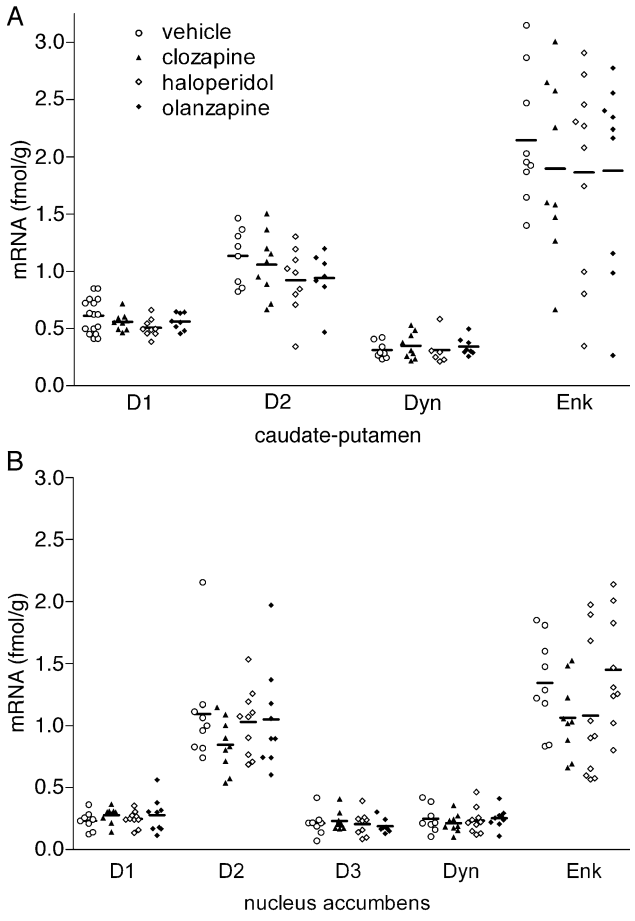

Fig. 5. Dopamine D1, D2, and D3 receptor (D1, D2, and D3), prodynorphin (Dyn), and preproenkephalin (Enk) transcript expression in the caudate-putamen (A) or nucleus accumbens (B) in rats treated with haloperidol-decanoate for 12 weeks followed by a 4 week washout, and another 4 weeks of additional treatment with vehicle, clozapine, haloperidol, or olanzapine. None of these molecules differed in expression levels between groups.

through graded alcohols and air-drying for 30 min. A cover slip with 60 μl of 1 million counts/min riboprobe, 75% formamide buffer (75% formamide, 10% dextran sulfate, 3× 300 mM NaCl/30 mM sodium citrate (pH 7.2), 50 mM Na_2HPO_4 (pH 7.4), 10 mM dithiothreitol, 1× Denhardt's solution, 100 μg/ml yeast tRNA), and 0.01 M dithiothreitol was placed on each slide. Slides were placed in a covered tray with filter paper saturated with 75% formamide. After overnight incubation at 55 °C, cover slips were removed and the slides were placed in 2× 300 mM NaCl/30 mM sodium citrate, pH 7.2, for 5 min, followed by RNAse (200 μg/ml in 10 mM Tris–HCl, pH 8.0/0.5 M NaCl) at 37 °C for 30 min and then washed as follows: 2× 300 mM NaCl/30 mM sodium citrate, pH 7.2, at room temperature for 10 min; 1× 300 mM NaCl/30 mM sodium citrate, pH 7.2, for 10 min at room temperature; 0.5× 300 mM NaCl/30 mM sodium citrate, pH 7.2, at 55 °C for 60 min; and 0.5× 300 mM NaCl/30 mM sodium citrate, pH 7.2, for 10 min at room temperature. The slides were dehydrated in graded ethanol solutions, air-dried, placed in X-ray cassettes, and apposed to Kodak XAR-5 film for 1–35 days depending on the probe under study.

2.6. Image and data analyses

Film was developed and used for image analysis (NIH Image 1.56), as previously described (Meador-Woodruff et al., 1997). Tissue background readings were subtracted from duplicate left and right side gray scale values from specific regions of interest. Gray scale values were converted into optical density and subsequently averaged, providing one mean value per region, per animal, for each probe. Optical density values were converted to units of bound radiation from a standard curve generated from a [^{14}C]microscale standard (Amersham Life Sciences, Amer-

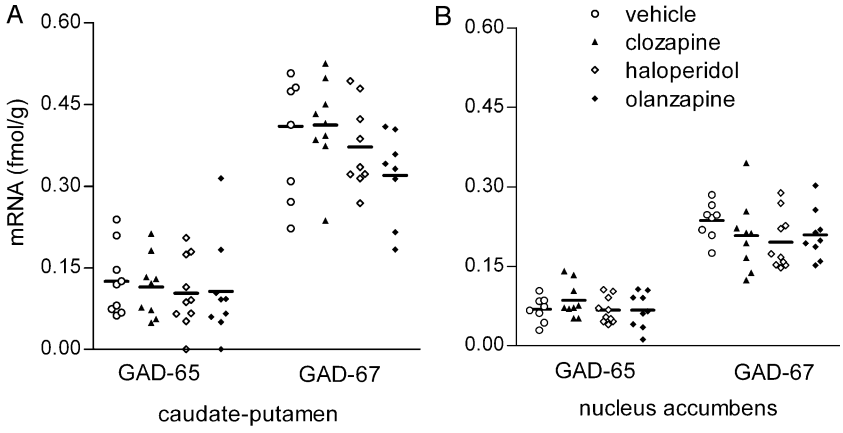

Fig. 6. Glutamic acid decarboxylase-65 (GAD-65) and GAD-67 transcript expression in the caudate-putamen (A) or nucleus accumbens (B) in rats treated with haloperidol-decanoate for 12 weeks followed by a four week washout, and four weeks of additional treatment with vehicle, clozapine, haloperidol, or olanzapine. None of these molecules differed in expression levels between groups.

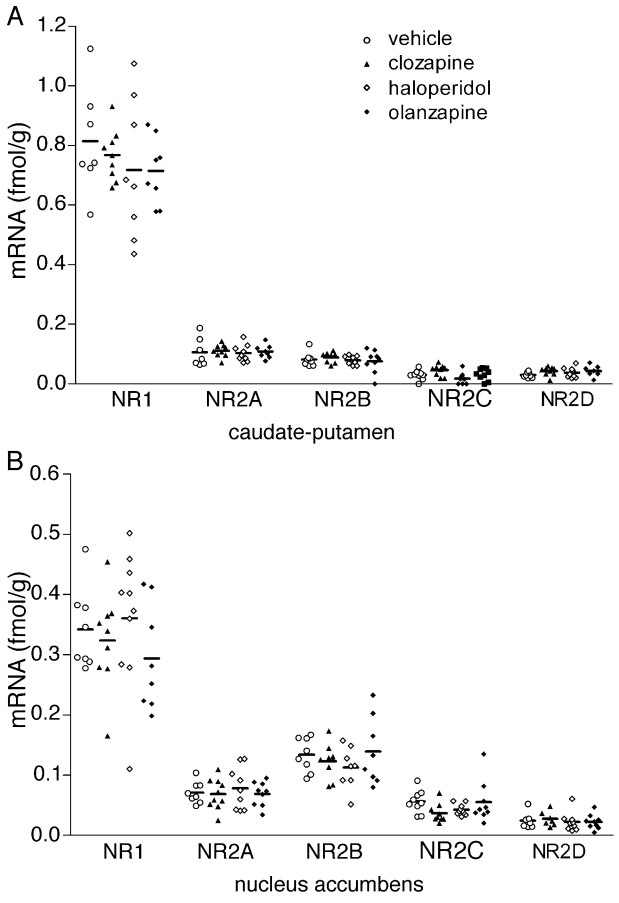

Fig. 7. NMDA receptor subunit-1 (NR1) and NMDA receptor subunits 2A-D (NR2A-D) transcript expression in the caudate-putamen (A) or nucleus accumbens (B) in rats treated with haloperidol-decanoate for 12 weeks followed by a 4-week washout, and 4 weeks of additional treatment with vehicle, clozapine, haloperidol, or olanzapine. None of these molecules differed in expression levels between groups.

sham UK) placed on each film (Downs and Williams, 1984; Williams, 1982). Transcript concentration, in turn, was determined from the amount of bound radiation, the specific activity of each batch of [^{35}S]uridine triphosphate, and the number of uracil residues contained in each probe sequence. All data are expressed as femtomoles mRNA per gram tissue (fmol/g).

2.7. Statistical analysis

Statistical analysis of vacuous chewing movement data before and after the treatment phase for each individual treatment group was performed with paired *t*-tests. Comparisons of the levels of vacuous chewing movements between groups before or after the 4-week treatment phase were performed using analysis of variance and post-hoc analysis with Tukey's honestly significant difference test. For all other data, statistical analyses were performed using analysis of variance, and when appropriate, post-hoc analyses were by Tukey's honestly significant difference test. For all tests, $\alpha = 0.05$.

3. Results

3.1. Vacuous chewing movements

Vacuous chewing movements observed in rats treated for 12 weeks with haloperidol-decanoate were measured at the end of weeks 1, 2 and 3 of the 4-week washout period. The 40 animals with the highest average number of vacuous chewing movements during the washout period were used for the treatment phase of the experiment (Fig. 1). Prior to the additional drug treatment, we did not detect any differences in vacuous chewing movements between groups of high vacuous chewing movement rats randomized into the four treatment groups ($F(3,35) = 1.21$, $P = 0.3$) (Fig. 2).

Following four additional weeks of treatment with vehicle, haloperidol, or olanzapine, post-treatment vacuous chewing movements were significantly lower compared to pretreatment vacuous chewing movements (vehicle: $t(8) = 4.84$, $P < 0.01$; haloperidol $t(8) = 2.30$, $P < 0.05$; olanzapine $t(9) = 4.31$, $P < 0.01$) (Fig. 2). We did not detect a significant difference between pre- and post-treatment vacuous chewing movements in the clozapine group ($t(8) = 0.3$, $P = 0.9$) (Fig. 2). Following the additional 4 weeks of treatment, we detected a main effect for drug treatment ($F(3,35) = 5.03$, $P < 0.005$). Post-hoc analysis indicated higher levels of VCMs in the clozapine group compared to the vehicle ($P < 0.01$), haloperidol ($P < 0.02$), and olanzapine ($P < 0.01$) treated groups (Fig. 3).

3.2. Gene expression in the striatum

Rat brain sections incubated with [^{35}S]riboprobes specific for neurotransmitter receptor subunit, enzyme, and peptide mRNAs demonstrated labeling with antisense, but

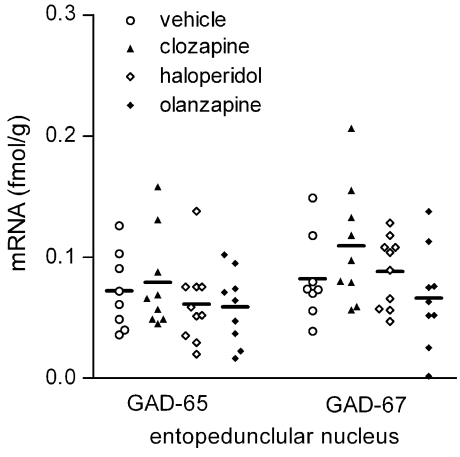

Fig. 8. Glutamic acid decarboxylase-65 (GAD-65) and GAD-67 transcript expression in the entopeduncular nucleus in rats treated with haloperidol-decanoate for 12 weeks followed by a 4-week washout, and 4 weeks of additional treatment with vehicle, clozapine, haloperidol, or olanzapine. None of these molecules differed in expression levels between groups.

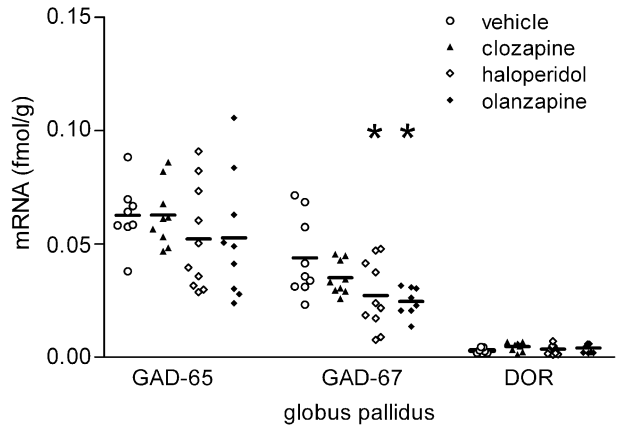

Fig. 9. Glutamic acid decarboxylase-65 (GAD-65), GAD-67, and δ-opioid receptor (DOR) transcript expression in the globus pallidus in rats treated with haloperidol-decanoate for 12 weeks followed by a 4-week washout, and 4 weeks of additional treatment with vehicle, clozapine, haloperidol, or olanzapine. GAD-67 mRNA was reduced in haloperidol and olanzapine-treated rats, while clozapine-treated rats were not decreased relative to controls. GAD-65 mRNA did not differ across groups (*$P<0.05$).

not sense, probes (data not shown). Transcripts for dopamine D1, D2 and D3 receptors, prodynorphin, preproenkephalin, GAD-65, GAD67, and the NMDA receptor subunits (NR1 and NR2A-D) were visualized and measured in the striatum (Fig. 4). We did not detect any treatment-specific effects for expression of dopamine D1, D2, D3 receptors, enkephalin, prodynorphin, GAD-65, GAD-67, NR1, or NR2A-D transcripts in the caudate-putamen or nucleus accumbens (Figs. 5–7).

3.3. Gene expression in the entopeduncular nucleus

Transcripts for GAD-65 or GAD-67 were detected in the entopeduncular nucleus. We did not detect any treatment-specific effects for expression of GAD-65 or GAD-67 transcripts in the entopeduncular nucleus (Fig. 8).

3.4. Gene expression in the globus pallidus

Transcripts for GAD-65, GAD-67, and the δ-opioid receptor were detected in the globus pallidus. We detected decreased GAD-67 mRNA in the globus pallidus following treatment of haloperidol-decanoate-induced orofacial

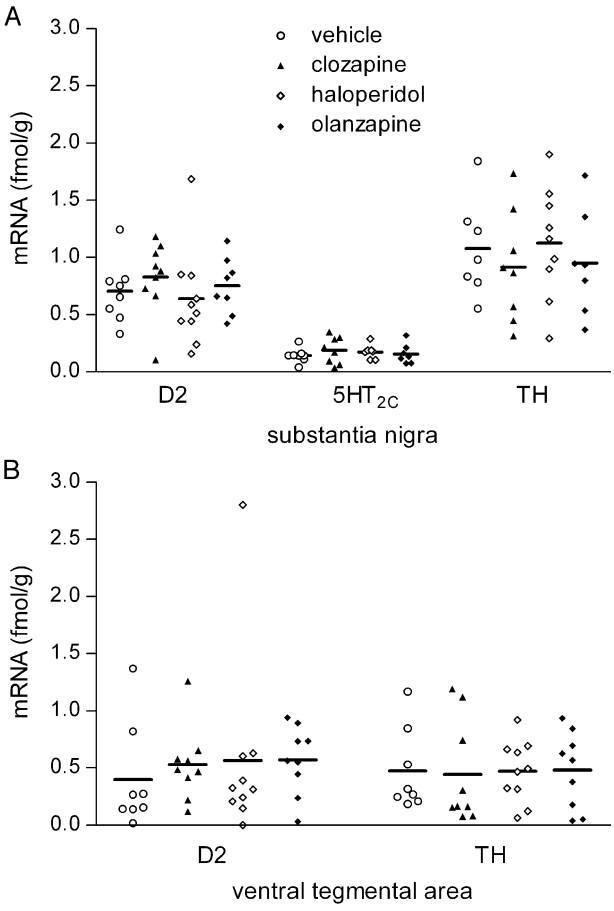

Fig. 11. Dopamine D2 receptor (D2), $5HT_{2C}$ receptor, and tyrosine hydroxylase (TH) transcript expression in the substantia nigra pars compacta (A) or ventral tegmental area (B) in rats treated with haloperidol-decanoate for 12 weeks followed by a 4-week washout, and 4 weeks of additional treatment with vehicle, clozapine, haloperidol, or olanzapine. None of these molecules differed in expression levels between groups.

movements ($F(3,34)=4.59$, $P<0.01$). Post-hoc analysis reveals that this decrease was due to olanzapine ($P<0.02$) and haloperidol ($P<0.04$), but not clozapine ($P=0.7$) treated rats having lower expression levels than in the vehicle treatment group. We did not detect any treatment-specific effects for transcript expression for either GAD-65 or the δ-opioid receptor in the globus pallidus (Fig. 9).

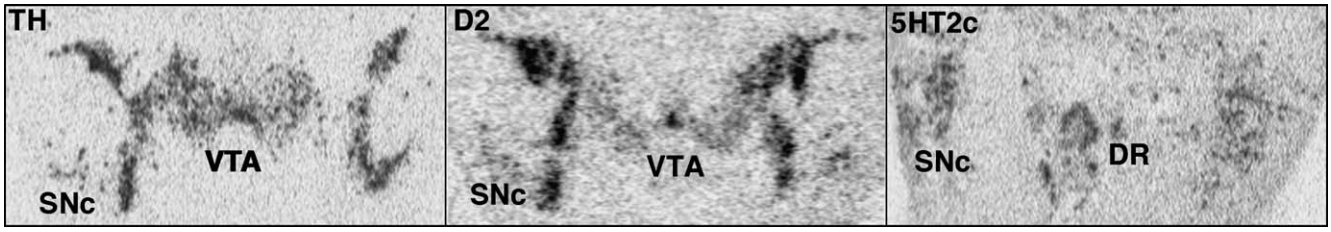

Fig. 10. Transcripts for tyrosine hydroxylase (TH) and the dopamine D2 receptor (D2) were detected in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) by in situ hybridization. Transcripts for the $5HT_{2C}$ receptor were detected in the substantia nigra (SN) and dorsal raphe (DR).

3.5. Gene expression in the substantia nigra/ventral tegmental area

Transcripts for tyrosine hydroxylase and the dopamine D2 receptor were detected in the substantia nigra pars compacta and ventral tegmental area (Fig. 10). Transcript for the 5-HT_{2C} receptor was also detected in the substantia nigra pars compacta (Fig. 10). We did not detect any treatment-specific effects for expression of dopamine D2 receptor or tyrosine hydroxylase in the substantia nigra pars compacta or ventral tegmental area (Fig. 11). We did not detect any treatment-specific effects for expression of 5-HT_{2C} receptor in the substantia nigra pars compacta (Fig. 11).

3.6. Summary of results

We found decreased haloperidol-induced vacuous chewing movements following additional treatment with vehicle, olanzapine, and haloperidol, but not clozapine. Post-treatment vacuous chewing movements in the clozapine group were significantly higher than in the other three groups. With the exception of GAD-67 mRNA expression in the globus pallidus, we did not detect any neurochemical changes in the striatum or its efferent targets.

4. Discussion

In this study, we evaluated the effects of 4 weeks of treatment with clozapine, olanzapine and haloperidol on vacuous chewing movements previously induced in rats with 12 weeks of haloperidol-decanoate treatment. Haloperidol-induced vacuous chewing movements were elevated in rats treated with clozapine compared to rats treated with vehicle, olanzapine, or haloperidol (Fig. 2). Previous studies have demonstrated elevated vacuous chewing movements following subchronic (3–4 weeks) and chronic (6–12 months) treatment with clozapine (20–27 mg/kg/day) (Ikeda et al., 1999; Kakigi et al., 1995; Rupniak et al., 1985; Yu et al., 1999). Vacuous chewing movements following subchronic treatment with clozapine were similar to vacuous chewing movements measured in haloperidol (1–2 mg/kg/day) treated rats (Ikeda et al., 1999; Yu et al., 1999). Levels of vacuous chewing movements following chronic treatment with clozapine were higher than controls, but lower than haloperidol-treated animals (Rupniak et al., 1985). Other studies have not detected changes in vacuous chewing movements following treatment with clozapine (Gunne et al., 1986; Marchese et al., 2002; See and Chapman, 1994). Variability in dose, route of administration, experimental design, behavioral methodology, and animal strain may account for these disparate results. One possible explanation of these dyskinesias is the timing of the vacuous chewing movement measurements. Yu et al. (1999) found significant differences in vacuous chewing movement levels 5- and 23-h post-injection in rats treated with either clozapine (25 mg/kg/day) or haloperidol (1 mg/kg/day) for 2 to 3 weeks, suggesting that the timing of the vacuous chewing movement measurements is critical in protocols whose route of administration is via injection. We measured vacuous chewing movements 24 h after the last injection, a time point that corresponds to the higher vacuous chewing movements in the clozapine group and the lower vacuous chewing movements in the haloperidol group reported in the study by Yu et al. Such an effect could account for the lower level of vacuous chewing movements in the haloperidol compared to clozapine-treated rats observed in the present study, since we measured vacuous chewing movements 24 h after drug administration.

One important difference between our study and previous reports of increased vacuous chewing movements following treatment with clozapine is that our protocol involved sequenced treatment, an approach analogous to switching a patient who has developed tardive dyskinesia from haloperidol to an atypical medication. Interestingly, haloperidol-induced vacuous chewing movements measured before and after treatment with clozapine were not significantly different, suggesting that clozapine did not permit vacuous chewing movements to extinguish (Fig. 2). However, the effects of sequenced treatment are difficult to interpret. While vacuous chewing movements induced in rats treated with either subchronic or chronic dosing of antipsychotics are phenotypically similar, they differ in several important ways. Only a subset of chronically treated rats develops a high level of vacuous chewing movements that may persist for many months, while subchronic treatment will induce a significant increase in vacuous chewing movements in most animals that persists for hours to days (Egan et al., 1996; Turrone et al., 2002). In addition, there are distinct differences in gene expression in chronic versus subchronic drug administration (Egan et al., 1996). Vacuous chewing movements measured following sequenced treatment putatively include both persistent- and acutely induced vacuous chewing movements, making it difficult to assess the relative contributions of the two drug treatments to the total number of vacuous chewing movements. One limitation of this study is that we did not include a group of vehicle control animals for the induction phase that went on to receive subchronic treatment with clozapine. Such a control might have permitted direct comparison of the levels of vacuous chewing movements following sequenced treatment (haloperidol-decanoate followed by clozapine) versus subchronic treatment alone (clozapine).

Haloperidol-induced vacuous chewing movements measured after treatment with haloperidol were significantly lower compared to haloperidol-induced vacuous chewing movement levels measured prior to the additional treatment (Fig. 2). Furthermore, we did not detect a difference in haloperidol-induced vacuous chewing movements following the additional treatment with haloperidol compared to vehicle controls, suggesting that vacuous chewing move-

ments extinguished despite additional treatment with haloperidol (Fig. 2). These results may be due to a masking effect of acute haloperidol administration. In one well-designed study, rats were treated with haloperidol-decanoate (28.5 mg/kg) every 3 weeks for 30 months, and following a 24-week washout period, the rats with moderate to high "tardive" haloperidol-induced vacuous chewing movements were given acute haloperidol doses (0.025–1.0 mg/kg) that attenuated vacuous chewing movements in a dose-dependent manner (Egan et al., 1996). In this experiment, rats treated acutely with 1 mg/kg of haloperidol had an average number of vacuous chewing movements per 2-min test interval less than 5, similar to our haloperidol-treated rats (Egan et al., 1996). Interestingly, acute haloperidol injections did not affect vacuous chewing movements induced by 7 days of treatment with haloperidol, demonstrating an important difference between vacuous chewing movements induced by chronic versus subchronic dosing.

In contrast to the effects of clozapine, haloperidol-induced vacuous chewing movements measured after olanzapine were decreased compared to before olanzapine treatment, suggesting that additional treatment with olanzapine permitted spontaneous recovery from chronic vacuous chewing movements. These results are consistent with the previous studies that have not detected increases in vacuous chewing movements following olanzapine treatment, despite considerable variation in experimental protocols (Gao et al., 1998; Moore et al., 1992; Roberts, 2001; Rosengarten and Quartermain, 2002). Vacuous chewing movement measurements have not been performed at multiple time-points post-drug injection in olanzapine-treated rats. However, unlike clozapine, none of the studies that have evaluated olanzapine have reported increases in vacuous chewing movements, suggesting that clozapine and olanzapine differentially influence stereotyped movements in analogous rat models of tardive dyskinesia.

Given that both are atypical antipsychotics, it was surprising that olanzapine and clozapine differed in their ability to extinguish vacuous chewing movements. Such an effect may be explained by differing 5-HT_{1A} receptor binding profiles for these drugs. Clozapine binds the 5-HT_{1A} receptor with an affinity at least an order of magnitude greater than olanzapine (Bymaster et al., 1996; Schotte et al., 1996). In addition, clozapine, but not olanzapine, has been identified as a partial agonist of 5-HT_{1A} receptors (Ichikawa et al., 2001). 5-HT_{1A} receptors are localized pre-synaptically in the raphe and post-synaptically in corticolimbic structures (Kia et al., 1996; Riad et al., 2000). This pattern of localization, together with the reports demonstrating regulation of dopamine release by serotonergic innervation, suggests that clozapine, but not olanzapine, may regulate dopamine release, and possibly stereotyped movements, via modulation of 5-HT_{1A} receptors (Cobb and Abercrombie, 2003).

Based upon the previous reports demonstrating persistently increased vacuous chewing movements in subsets of rats treated chronically with haloperidol-decanoate, we predicted persistently elevated haloperidol-induced vacuous chewing movements in the vehicle control group after the treatment phase of the experiment. However, the most effective reduction in the level of vacuous chewing movements was observed in the vehicle group after the treatment phase, since vacuous chewing movements were significantly extinguished by 8 weeks after the last haloperidol-decanoate injection (4 weeks of washout plus 4 weeks of vehicle injections) (Fig. 2). One possibility is that we may not have permitted enough time following the induction phase of the experiment for the rats to develop persistent vacuous chewing movements. Other groups have waited for up to 12 months prior to assigning chronically treated rats to "high" and "low" vacuous chewing movement groups (Egan et al., 1996; Hashimoto et al., 1998; Roberts, 2001). We sorted the chronically treated rats based upon vacuous chewing movement measurements obtained only 1 to 3 weeks after cessation of haloperidol-decanoate treatment. Thus, identification of rats exhibiting "high" vacuous chewing movements may have included rats with higher acute vacuous chewing movements that did not go on to develop persistent vacuous chewing movements. This may also explain the apparent decrease in vacuous chewing movements following treatment with haloperidol and olanzapine.

Numerous studies have detailed altered gene expression in the striatum and its efferent targets following subchronic or chronic antipsychotic treatment (Andreassen and Jorgensen, 2000; Jeste et al., 1998; Turrone et al., 2002). The effect of sequenced treatment, however, an approach analogous to switching a patient who has developed tardive dyskinesia from haloperidol to an atypical medication, has not been evaluated. We detected decreased GAD-67 transcript expression in the globus pallidus in rats given intramuscular haloperidol-decanoate (12 weeks) followed by either haloperidol or olanzapine (4 weeks), compared to control animals that received haloperidol-decanoate for 12 weeks followed by vehicle (4 weeks). GAD-67 mRNA expression in the globus pallidus in the clozapine treatment group was not significantly different from the haloperidol, olanzapine, or control groups. A decrease in GAD-67 transcript expression may reflect attenuated GABAergic neurotransmission in the indirect pathway, possibly resulting in disinhibition of the substantia nigra, pars reticulata or subthalamic nucleus. Vacuous chewing movements were extinguished in the vehicle, haloperidol and olanzapine, but not clozapine, treatment groups. Thus, no conclusions may be drawn regarding the interaction between vacuous chewing movements and gene expression in the globus pallidus following sequenced treatment. In addition, we did not detect any significant correlations between vacuous chewing movements and transcript expression in the globus pallidus.

While the changes we detected in GAD-67 expression may be due to a type I statistical error given the number of statistical tests performed in this study, there is a small but

growing body of evidence implicating altered gene expression in the globus pallidus of rats challenged with antipsychotics. Decreased $GABA_A$ receptor density was demonstrated in the globus pallidus following 6 months of treatment with haloperidol or sertindole, but not olanzapine (Sakai et al., 2001). In this study, changes in GAD-67 expression in the globus pallidus were not detected. Other groups have evaluated the effects of subchronic treatment with antipsychotics on GABAergic neurotransmission. GAD mRNA expression in the globus pallidus was increased after 4 weeks of treatment with clozapine, but not haloperidol, while a more recent study reported GAD-67 mRNA expression in the globus pallidus was increased with haloperidol, and decreased with clozapine (Delfs et al., 1995a,b; Mercugliano et al., 1992). Studies of acute antipsychotic treatment are contradictory as well. Acute subcutaneous administration of haloperidol, but not clozapine, stimulated increased GABA release in the globus pallidus measured by microdialysis (Drew et al., 1990). When administered by reverse microdialysis directly into the globus pallidus, clozapine induced a decrease in GABA release, while haloperidol had no effect (See and Berglind, 2001). These disparate results are likely secondary to differences in route of administration. Nevertheless, regulation of GAD-67 transcript expression appears to be sensitive to differential treatment with typical and atypical antipsychotics.

Despite markedly differing effects on vacuous chewing movements, we did not detect any changes in gene expression with olanzapine, clozapine, or haloperidol treatment outside of the globus pallidus. Numerous studies have described increases in dopamine D2 receptor, preproenkephalin, and prodynorphin expression in the striatum of rats treated chronically with haloperidol (Andreassen and Jorgensen, 2000; Jeste et al., 1998; Turrone et al., 2002). Since all of the rats in the treatment phase of our experimental protocol were previously challenged with haloperidol-decanoate for 12 weeks (induction phase), alterations in dopamine D2 receptor, preproenkephalin, and prodynorphin expression induced by chronic haloperidol treatment would only be apparent if compared to an age-matched control group of animals that did not receive treatment with haloperidol-decanoate in the induction phase.

The absence of detected alterations in transcript expression in our sequenced treatment experiments may be related to the way we quantified gene expression. Alterations in gene expression following typical versus atypical antipsychotic treatment may be at the level of translation, receptor activation or intracellular signaling, and thus would not be detected by transcript studies. In addition, other groups of molecules found in the various neurotransmitter systems that interface with the striatal efferent pathways may be regulated by antipsychotics. For example, striatal glutamate transporter expression and aspartate uptake were decreased in rats treated with antipsychotics, suggesting that regulation of glutamatergic neurotransmission in the striatum and its efferent targets may occur in classes of molecules other than glutamate receptors (De Souza et al., 1999; Delfs et al., 1995a).

This study demonstrates spontaneous remission of vacuous chewing movements with olanzapine, haloperidol, and vehicle, but not clozapine, following treatment of haloperidol-induced vacuous chewing movements in Sprague–Dawley rats. Somewhat surprisingly, we did not find any neurochemical changes in the striatum and its efferent pathways except for a decrease in GAD67 in the globus pallidus. These findings highlight an important difference in the effects of atypical antipsychotics and contribute to a growing body of evidence pointing toward alternative hypotheses for the pathophysiology of vacuous chewing movements.

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