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ORIGINAL INVESTIGATION

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Pharmacological and neurochemical differences between acute and tardive vacuous chewing movements induced by haloperidol

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Abstract Late onset vacuous chewing movements (VCMs) from chronic neuroleptic treatment have been used as a rat model of tardive dyskinesia (TD). Early onset VCMs have also been observed, raising questions about the validity of this model. To assess the relationship between these two types of VCMs, pharmacological and neurochemical properties of early and late onset VCMs were compared. "Acute" VCMs were induced by daily intraperitoneal injections for 1–21 days. "Tardive" VCMs were induced by intramuscular injections of haloperidol decanoate every 3 weeks for 30 weeks followed by a 24-week withdrawal period. Suppression was attempted for both types of VCMs using several doses of intraperitoneal haloperidol. Striatonigral activation was assessed by measuring mRNA expression levels of the neuropeptides dynorphin and substance P using in situ hybridization histochemistry. Enkephalin mRNA was also measured as an index of striatopallidal activation. The results indicate that acute VCMs cannot be suppressed with increased doses of haloperidol and are associated with reduced dynorphin and substance P. This profile is similar to that seen with an animal model of parkinsonism. Tardive VCMs, in contrast, were markedly suppressed by haloperidol. They have previously been shown to be associated with increased striatonigral activation as indicated by increased dynorphin mRNA. Enkephalin mRNA was elevated following both short and long term treatment. Although superficially similar, acute and tardive VCMs appear to have different pharmacological and neurochemical profiles, suggesting they are related to acute extrapyramidal side effects and tardive dyskinesia, respectively.

Key words Vacuous chewing movements · Haloperidol · Tardive dyskinesia · Substance P · Dynorphin · Enkephalin

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Introduction

The development of antipsychotic medications has been a dramatic breakthrough for the treatment of psychoses. Use of these drugs has been limited, however, by a number of undesirable side effects. Tardive dyskinesia (TD), a potentially disfiguring and sometimes irreversible movement disorder, is particularly problematic due to its high incidence and the lack of effective treatment. Clozapine, an "atypical" neuroleptic, rarely causes TD. Unfortunately, its use has also been limited by complications such as agranulocytosis and sedation. Other "atypical" agents are in development, but it is unclear whether or not they will cause TD. The syndrome of vacuous chewing movements (VCMs) is a rodent model that has been employed to investigate the pharmacology and neurobiology TD. VCMs develop gradually during long term administration of neuroleptics and are reminiscent of the orobuccal movements of TD. Concerns about validity, however, have limited use of this model, particularly in the development of new "atypical" agents. Some investigators have suggested VCMs are a better model of acute extrapyramidal symptoms (EPS), such as dystonia, than of TD (Rupniak et al. 1986).

The idea that VCMs may be an acute EPS rather than a dyskinesia is based on observations that VCMs can develop during short term neuroleptic treatment (Rupniak et al. 1985, 1986). An essential feature of TD is that its onset is delayed for months to years after the initiation of treatment (APA Task Force 1992). Most studies of the

VCM syndrome have found that its onset is likewise delayed by 8–15 weeks (Waddington 1990), the rat equivalent of several years in man (Tamminga et al. 1990). This relative temporal homology between VCMs and TD appears to be a significant strength of the model. A delayed onset of VCMs is typically observed when neuroleptics are administered in drinking water or by intramuscular injection with slow release preparations (e.g. haloperidol decanoate). It appears that VCMs may also develop rapidly, particularly following intraperitoneal (IP) or subcutaneous (SC) injection (Steinpreis et al. 1993). This acute onset suggests that VCMs are similar to acute EPS, typically seen shortly after initiation of neuroleptic treatment (Rupniak et al. 1983, 1985, 1986). The nature of the relationship between "acute" and "tardive" VCMs is unclear (Waddington and Malloy 1987) . If VCMs are parkinsonian in nature, their use as a model of TD would be limited.

Hyperkinetic dyskinesias and hypokinetic disorders, such as acute EPS, are distinguishable in several ways. First, they respond differently to acute D_2 blockade. A number of studies have shown that TD is most effectively suppressed by increased doses of neuroleptics (Jeste et al. 1988; APA Task Force 1992). Acute EPS, on the other hand, can be induced by such treatments. Effects of increased neuroleptic dose on "acute" and "tardive" VCMs could distinguish the tardive versus parkinsonian nature of these movements. Unfortunately, prior studies of VCM suppression with neuroleptics have been inconclusive, most likely because of methodological differences (Gunne et al. 1982; Waddington et al. 1986a,b, 1990; Stoessl et al. 1989; Egan et al. 1995a; see Discussion). A second difference between dyskinesias and acute EPS is their underlying neurobiology. Hyperkinetic movements appear be related to increased activation of the D_1-mediated (or "direct") striatonigral pathway. Hypokinetic disorders, on the other hand, are associated with reduced activation of the direct pathway, and/or increased activation of the D_2 mediated "indirect" pathway (Crossman 1990; DeLong 1990; Gerfen et al. 1990, 1991). Several peptides are co-expressed in these two pathways: dynorphin and substance P in the former, and enkephalin in the latter. Messenger RNA levels of these peptides have been used as markers of neuronal activation. Thus, assessing mRNA levels of dynorphin, substance P, and enkephalin in acute and tardive VCMs may provide a clue regarding their relationship to acute EPS and dyskinesias.

In rats with tardive VCMs, increased expression of dynorphin mRNA suggests that activation of the direct pathway is increased (Egan et al. 1994a). This indicates that tardive VCMs share features with hyperkinetic disorders. The level of activation of the direct pathway in "acute" VCMs is less certain. While a number of studies have looked at changes in dynorphin and substance P mRNA following various doses of neuroleptics, none has concomitantly assessed chewing movements.

We compared "tardive" and "acute" VCMs in two ways. First, response to acute injections of neuroleptics was examined. Acute EPS should remain unchanged or increase, while tardive movements should be suppressed. As a corollary to this experiment, we first sought to describe dose/response characteristics and the duration of acute VCMs. Second, activation of the direct and indirect striatal efferent pathways was assessed using in situ hybridization histochemistry to quantitate levels of mRNA expression for the neuropeptides dynorphin, substance P and enkephalin in rats with acute VCMs. We hypothesized that acute VCMs would demonstrate similarities to EPS but tardive VCMs would not. These findings would support the validity of the tardive VCM syndrome as a model for TD in humans.

Materials and methods

Animals and drug treatment

Drug-naive, male Sprague-Dawley rats initially weighing 175–200 g were housed in groups of three with free access to food and water, a 12-h light-dark cycle (with lights on at 7 a.m. and off at 7 p.m.), and constant temperature (25°C). Rats were acclimatized for 1 week before experimentation. Acute VCMs were induced using intraperitoneal (IP) injections of haloperidol solutions prepared fresh daily. Haloperidol (Sigma, St Louis, Mo.) was dissolved in glacial acetic acid (40 mg/ml), partially neutralized with 0.13 ml 1 M NaOH per 1 ml solution, and diluted with distilled water. Final concentrations varied from 0.025 to 2.0 mg/kg and are described in the figures. Vehicle solutions were prepared identically except that haloperidol was omitted. Animals were injected IP with a volume of 1.0 ml/g body weight. All procedures complied with the NIH Guide for the Care and Use of Laboratory Animals.

Two cohorts were employed to characterize acute VCMs. The first was used to assess 1) the relationship between dose and VCM severity, 2) VCM persistence, and 3) suppressibility of VCMs with increased doses. In the dose/response study, 0.025–2.0 mg/kg haloperidol was administered IP (n=8–9/group), and VCMs counted after 1 h. Five groups continued to receive injections (veh, 0.025, 0.1, 0.4, or 1.0 mg/kg haloperidol) daily for 7 days to assess persistence. To examine persistence over a single day, VCMs were counted 1, 3, 6, and 23 h after injections (on day 4) in two groups (0.025 and 0.4 mg/kg groups). To evaluate suppressibility of acute VCMs, doses were doubled from days 8–10, and VCMs again counted 1 h after injections.

A second cohort was used to assess expression of dynorphin, substance P, and enkephalin mRNA in striatonigral and striatopallidal neurons. Animals received 0.4 mg/kg IP of haloperidol or vehicle daily for 21 or 22 days. This length of treatment was initially selected to look for evidence of long term tolerance or progression of VCM severity. VCMs were counted 1 h after injections every other day. On day 22, haloperidol treated animals received a final injection of either haloperidol (1-h group, n=15) or vehicle (24-h group, n=10); all vehicle treated animals (n=10) received a final injection of vehicle. The 24-h group (which received its last injection of active drug 24 h prior to brain removal) was used as an additional control to assess acute effects of haloperidol (1-h group) following 21 daily injections. Deep anesthesia was induced with 40 mg/kg (IP) pentobarbital. Euthanasia was accomplished using intracardiac infusion with phosphate buffered saline (4°C). Brains were quickly removed, frozen in powdered dry ice, and stored at -70° C prior to sectioning.

For studies of tardive VCMs, a group of 75 male Sprague-Dawley rats were used. These animals initially weighed 140–160 g and were housed in groups of two or three as described above. Animals received intramuscular (IM) haloperidol decanoate 28.5 mg/kg (McNeil Pharmaceuticals, Spring House, Pa.), the equivalent of 1.0 mg/kg per day of unconjugated haloperidol (n=60), every 3 weeks for 30 weeks, or vehicle (kindly donated by

McNeil Pharmaceuticals, Spring House, Pa.) (n=15). VCMs were counted 1 day before each haloperidol injection, and every 6 weeks during a 24-week withdrawal period. Ratings from all 14 rating sessions were summed after the final rating session. A subgroup of 24 haloperidol-treated rats with moderate to high total VCM scores was selected. Rats that did not develop VCMs were not included. The selected animals were randomly assigned to one of four groups (n=6/group). VCMs were counted at 11 a.m. Acute IP injections of haloperidol (0.25–1.0 mg/kg) were given and VCMs again counted 1 h later.

Behavioral measures

VCMs were counted during 2-min periods between 10 a.m. and 2 p.m. in the same room in which animals were housed. Rats were transferred from their home cages to uncovered 20×30×40 cm plastic cages located on a rotating platform, allowing raters to observe mouth movements at all times. Habituation periods were not employed since ratings before and after habituation are highly correlated (Egan et al. 1995b). All VCM ratings were performed by raters blind to treatment and previous ratings. Inter-rater reliability was highly significant (F=12.1, df=31,32, ICC=0.85, P<0.0001). As previously noted, two types of jaw movements were observed (Gunne 1982). Intermittent chewing movements which occurred in isolation and were unrelated to grooming, gnawing, or eating were each counted as one movement. A second type of movement, bursts of chewing, consisted of two to six individual VCMs in rapid succession and was usually associated with jaw tremors. These burst episodes were counted separately but were not included in the data analysis due to the low numbers observed. Tremorous movements were not included in VCM scores. Tongue protrusions were also observed but not counted.

In situ hybridization histochemistry

In situ hybridization was performed as previously described (Hurd and Herkenham 1992) for dynorphin, substance P, and enkephalin mRNA. Briefly, frozen coronal sections (20 μm thick) were cut through the striatum, thaw-mounted onto gelatin-subbed slides, and stored at —70°C until further processing. Sections were subsequently fixed by immersion in 4% formaldehyde in phosphate-buffered saline (PBS) for 5 min at 25°C, rinsed twice in PBS, treated with 0.25% acetic anhydride, in 0.1 M triethanolamine/0.09% saline (pH 8.0) for 10 min, dehydrated serially in 70%, 80%, 95%, and absolute ethanol, delipidated in chloroform for 5 min, rinsed in absolute and 95% ethanol, and air dried. Synthetic 48-base oligodeoxyribonucleotide probes directed against prodynorphin (bases encoding 862–909; Civelli et al. 1985), protachykinin (substance P, bases encoding 124–171; Krause et al. 1987) and preproenkephalin (bases encoding 388–435; Yoshikawa et al. 1984), were labeled at the 3′ end using alpha-[^{35}S]dATP (>1000 Ci/mmol, New England Nuclear, Boston, Mass.), terminal deoxynucleotidyl transferase (25 U/μl, Boehringer-Mannheim, Mannheim, Germany), and tailing buffer (Bethesda Research Labs, Gaithersburg, Md.). Sections were hybridized overnight at 37°C with 5×10^{-5} cpm of labeled probe in buffer containing 4× standard saline citrate (SSC; 1×=0.15 M NaCl/0.015 M sodium citrate, pH 7.2), 50% (v/v) formamide, 10% (w/v) dextran sulfate, 0.02% Ficoll, 0.02% bovine serum albumin, 500 μg/ml sheared single-stranded salmon sperm DNA (Sigma, St Louis, Mo.), 250 μg/ml yeast tRNA (Bethesda Research Labs) and 0.1 M dithiothreitol. Sections were washed in four 15-min rinses of 2× SSC containing 50% formamide at 40°C, two 30-min rinses of 1× SSC at 25°C, one brief rinse of distilled water, and dried. Slides, ^{14}C standards (American Radiochemicals, St Louis, Mo.) and ^{35}S-impregnated brain paste standards of known radioactivity and tissue content were placed in X-ray cassettes, apposed to film for 3–18 days, and developed for 4 min at 20°C.

Film images were digitized using a MacIntosh-based image analysis system (IMAGE, Wayne Rasband, NIMH), and optical density of the areas of interest was determined by tracing the structure with a hand-held cursor. Striatal sections extended from 2.7 mm to 1.6 mm anterior to bregma (Paxinos and Watson 1986). The striatum was divided into dorsolateral (DLS) and ventromedial (VMS) aspects (see Egan et al. 1994a). Optical density for striatal subregions and nucleus accumbens (ACC) was measured bilaterally in two sections per animal, converted to DPMs per mg wet weight of tissue using the standard curve generated by co-exposed ^{14}C-standards (calibrated against brain paste ^{35}S standards), and averaged.

Statistics

Behavioral data from acute VCM studies were evaluated by analysis of variance (ANOVA) followed by post hoc comparisons using Fisher's protected LSD. For repeated ratings, repeated measures analysis of variance (rmANOVA) was used. Subsequently, individual rating sessions were assessed for group effects using ANOVAs followed by Fisher's protected LSD. To test for suppression of acute VCMs following doubling of doses, a rmANOVA was employed including all haloperidol-treated groups. A second rmANOVA was performed including only data from the three highest dose groups (0.1–1.0 mg/kg). To test the hypothesis that acute haloperidol injections suppressed tardive VCMs, a rmANOVA was employed including VCM scores before and after acute injections. For analyses that violated the assumption of homogeneity of variances where means correlated with variances, log transformations were first performed.

For neurochemical data, differences between groups (vehicle, chronic haloperidol after 1 h and chronic haloperidol after 24 h), were assessed using ANOVA followed by Fisher's protected LSD. When this analysis was not significant, both 1 h and 24 h groups were combined and compared to vehicle treated groups to look further for a neuroleptic effect.

Results

Acute VCMs

A single IP injection of haloperidol induced VCMs at doses of 0.1 mg/kg and above (Fig. 1) (group effect: F=17.9, df=6,54, P=0.0001). While these VCMs were seen after 1 h, they were apparently short lived. Follow-

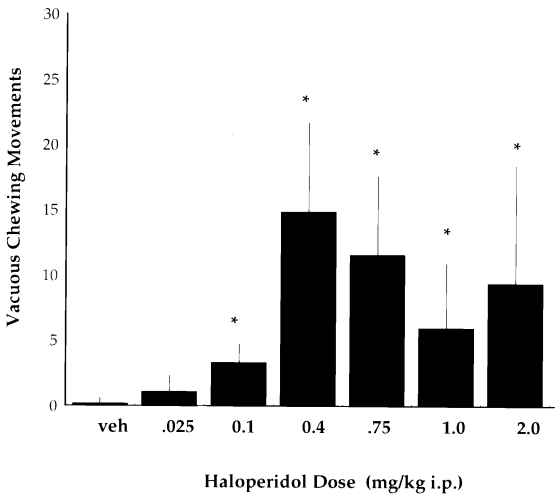

Fig. 1 Dose-response curve for acute IP injections of haloperidol. Animals were given IP injections of vehicle or varying doses of haloperidol (*P<0.05 compared to vehicle) (n=8–9/group)

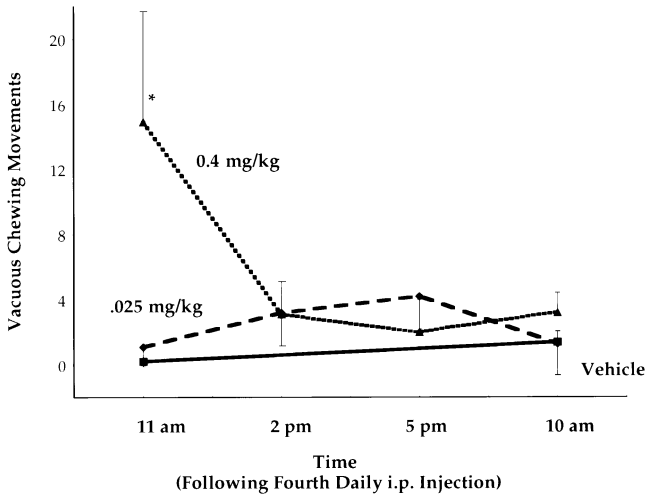

Fig. 2 Duration of VCMs following a single IP injection of haloperidol. Rats treated with 0.025 and 0.4 mg/kg IP were rated at 11 a.m., and again after 3, 6, and 23 h (n=9/group). *P<0.05, compared to vehicle

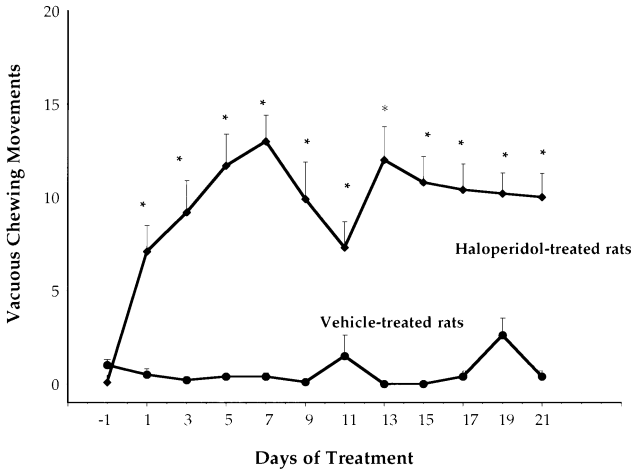

Fig. 3 VCMs persist during 3 weeks of daily IP haloperidol injections. *P<0.05, compared to vehicle

ing the fourth daily injection, acute VCMs were elevated 1 h after 0.4 mg/kg IP but fell towards baseline levels after 3 h (Fig. 2). After 23 h, VCM scores were at baseline levels (time effect: F=14.2, df=1, P=0.001; dose effect: F=33.5, df=2, P=0.0001; and dose by time interaction: F=20.9, df=2, P=0.0001, respectively). Continued daily IP treatment over 7 days showed that VCMs could be elicited repeatedly by haloperidol doses of 0.1 mg/kg and above compared to vehicle treatment (group effect: F=21.2, df=4, P=0.0001). The lack of a time effect (F=1.6, df=4, P=0.2), however, suggest that VCMs did not markedly increase over this 7-day period (data not shown). Treatment for 21 days with 0.4 mg/kg in a second cohort (Fig. 3) continued to elicit acute VCMs (drug effect: F=41.9, df=1, P=0.0001). Again, the lack of a time effect (F=0.11, df=10, P=0.65) indicated that scores did not markedly change during this period.

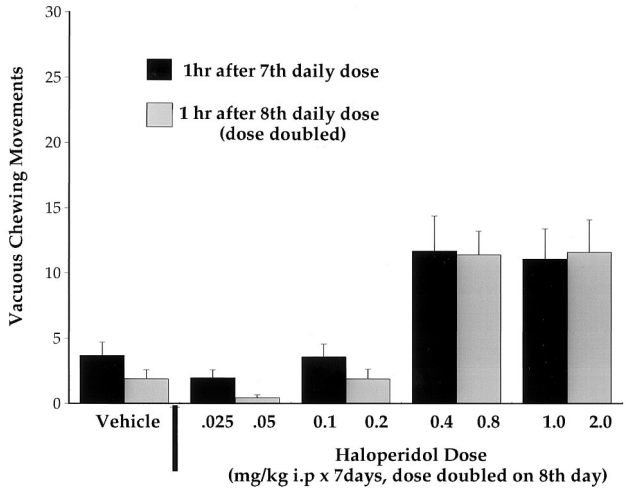

Fig. 4 Following 7-day treatment with a fixed dose of IP haloperidol, rats received double the usual dose (n=8–9/group). VCMs were counted 1 h later. No suppressive effect was found for all groups or only those groups treated with high doses of haloperidol (0.1, 0.4, and 1.0 mg/kg). The 0.4 and 1.0 mg/kg groups had increased VCM scores compared to vehicle

Attempts to suppress VCMs by doubling haloperidol doses on day 8 were not effective (time effect: F=1.9, df=1, P=0.17) (Fig. 4). No significant reductions in chewing movements were noted whether all groups were included in a rmANOVA or only groups that received the three highest doses (0.1–1.0 mg/kg; time effect: F=0.88, df=1, P=0.35). Continued treatment for 4 days with these increased doses did not alter VCM counts further. Similar to day 1 (Fig. 1), on days 7 and 8 a significant effect of haloperidol dose was again found (F=30.7, df=4, P=0.0001) with doses of 0.1 mg/kg and above elevating VCM scores compared to vehicle levels.

Tardive VCMs

Over the initial 30-week treatment period, the haloperidol group showed a gradual increase in VCMs compared with vehicle (treatment effect: F=45.4, df=1, P=0.0001; time effect: F=9.9, df=13, P=0.0001; treatment by time interaction: F=3.7, df=13, P=0.0001) (Fig. 5). VCM scores remained significantly elevated during the 6-month withdrawal period. Before acute haloperidol challenge, there were no differences in VCM scores between groups receiving different haloperidol doses (F=0.57, df=3, P=0.64). Acute IP injections of haloperidol markedly reduced VCM scores (Fig. 6) (suppression effect: F=5.6, df=1, P=0.04). Neither dose effect (F=0.19, df=3, P=0.90) nor dose by time interaction (F=1.5, df=3, P=0.27) was significant, however.

mRNA levels in acute haloperidol group

Dynorphin levels were generally reduced in haloperidol treated animals compared to controls. A significant ef-

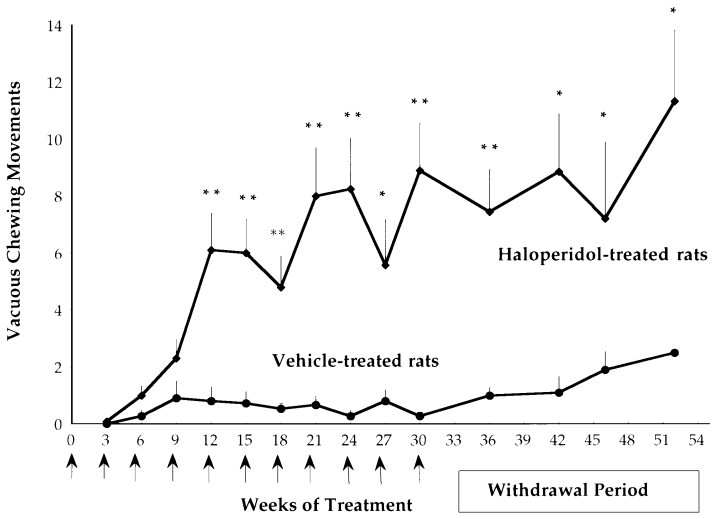

Fig. 5 Tardive VCMs in rats injected every 3 weeks (*arrows*) for 30 weeks with haloperidol decanoate. Haloperidol significantly increases VCMs compared to placebo. This effect is delayed by 12 weeks and persists during a 6-month withdrawal period. *P<0.05, **P<0.001 compared to vehicle

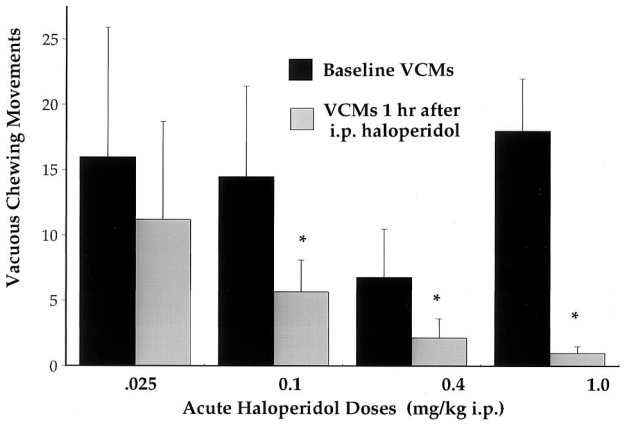

Fig. 6 Suppression of tardive VCMs with acute IP injections of haloperidol. VCMs were counted at 11 a.m. Different doses of haloperidol were injected and VCMs were again counted 1 h later. A marked reduction in VCM counts was observed. No dose effect or dose by time interaction was seen

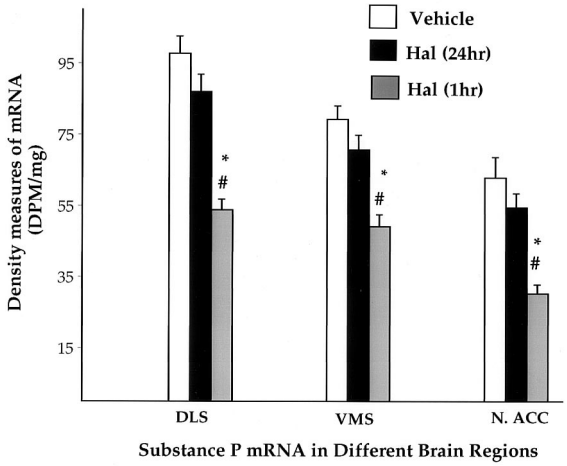

Fig. 8 Substance P mRNA expression levels (DPM/mg) in vehicle and two haloperidol-treated groups. Significant group effects were seen in all regions (see text) (P<0.05 compared to *control and #24-h groups)

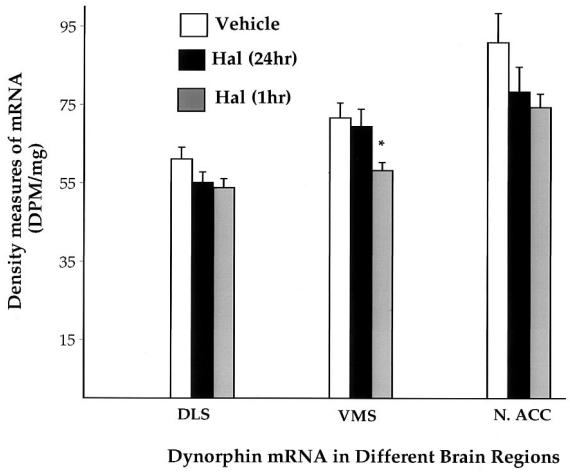

Fig. 7 Dynorphin mRNA expression levels (DPM/mg) in vehicle and two haloperidol-treated groups. Significant group effects were seen in the VMS (P<-0.05 compared to *control and #24-h groups)

fect of group was seen in the VMS (F=5.1, df=2, P=0.01) but not in the DLS (F=2.0, df=2, P=0.15) or ACC (F=2.4, df=2, P=0.10, respectively). Reductions were most prominent in the 1-h group, which had received a final haloperidol injection 1 h before brain removal (Fig. 7). Combining the two haloperidol treated groups, a significant effect of treatment was also seen in the ACC (F=4.55, df=1, P=0.04), while a trend was seen in the DLS (F=3.49, df=1, P=0.07).

Levels of substance P mRNA were also reduced by haloperidol treatment. Significant group effects were seen in all regions (DLS, F=24.7, P=0.0001; VMS F=15.4, P=0.0001; and ACC F=11.9, P=0.0003). Changes were again most pronounced 1 h, rather than 24 h, after the final injection of haloperidol (Fig. 8).

Enkephalin mRNA was markedly increased in both haloperidol-treated groups. Significant group effects were seen in all regions (DLS, F=16.82, df=2, P=0.0001; VMS F=14.5, df=2, P=0.0002; and ACC F=5.46, df=2,

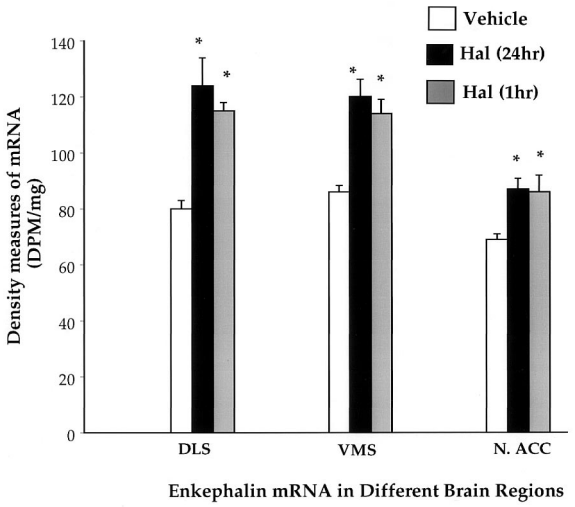

Fig. 9 Enkephalin mRNA expression levels (DPM/mg) in vehicle and two haloperidol-treated groups. Significant group effects were seen in all regions (*P<0.05 compared to control)

P=0.01). Changes were again most pronounced 1 h, rather than 24 h, after the final injection of haloperidol (Fig. 9).

Discussion

The results suggest that, in spite of their apparent similarity, acute and tardive VCMs are pharmacologically and neurochemically distinct. Acute VCMs were seen 1 h after IP injections of haloperidol. Severity increased with increasing doses, peaking at 0.4 mg/kg. Following seven daily injections, they could not be suppressed by doubling haloperidol doses. Acute VCMs, furthermore, were associated with reduced dynorphin and substance P mRNA expression , suggesting reduced striatonigral activation. In contrast, tardive VCMs developed gradually and persisted during an extended withdrawal period, as brain haloperidol levels become very low (Egan et al. 1994a). Tardive VCMs were suppressed by acute IP injections of haloperidol. Late onset VCMs have previously been associated with increased mRNA for dynorphin with no change in substance P, implicating increased striatonigral activation (Egan et al. 1994a). Taken as a whole, these data suggest that tardive VCMs are similar to TD, while acute VCMs share features with acute EPS, or parkinsonism.

Effects of acute increases in neuroleptic dose have been examined previously for tardive but not acute VCMs. These reports have been inconsistent, most likely because of methodological differences. Waddington et al. (1986 a,b) failed to suppress VCMs using haloperidol (0.75 mg/kg SC), but low basal rates (1.9 per 5 min) suggest tardive VCMs had not yet developed fully. Gunne et al. (1982) found that 2 mg/kg IP haloperidol reduced VCMs in animals with cortical ablations, a procedure which also induces VCMs. A third study (Stoessl et al. 1989), similar to ours, found that raclopride (0.5 mg/kg IP) reduced tardive VCMs; a fourth study failed to suppress VCMs with long term increases in haloperidol decanoate dose (Egan et al. 1995a). When methodological differences are taken into account, these studies support the current findings that tardive VCMs can be temporarily suppressed by neuroleptics. In this respect, tardive VCMs are similar to TD in humans, which can also be suppressed with increased neuroleptic dose (Jeste et al. 1988; APA Task Force 1992).

Acute VCMs, in contrast, were induced, rather than suppressed, by IP injections of haloperidol. Acute VCMs increased with doses up to 0.4 mg/kg (Fig. 1). Initially, VCMs appeared to fall with higher doses (e.g. 1.0 mg/kg scores were lower than those for 0.4 mg/kg); further studies, however, found similar scores for these high doses (data not shown), suggesting that a plateau is reached. Regarding suppression, the high VCM counts induced by doses of 0.4 mg/kg and above were clearly not suppressed with increased doses (Fig. 4). Furthermore, acute VCMs abated within hours after the fourth daily dose. In contrast, tardive VCMs vary little throughout the day following a 6-month withdrawal from decanoate injections (Egan et al. 1995b). The time course for acute VCMs is reminiscent of that for acute EPS, such as dystonia, which remit rapidly, after treatment is withdrawn (e.g. see Rupniak et al. 1986 for discussion).

Neurochemical results support the idea that acute and tardive VCMs are distinct. In general, the mRNA results are consistent with previous studies; for example, substance P mRNA and/or peptide levels have been reported to be reduced by a number of different neuroleptic treatment paradigms for 1–14 days (Hong et al. 1978; Hanson et al. 1981; Bannon et al. 1986, 1987; Nylander and Terenius 1986; Li et al. 1987; Cruz and Beckstead 1988; Radke et al. 1988; Shibata et al. 1990; Pollack and Wooten 1992). The effects of neuroleptics on dynorphin levels and mRNA expression are less clear. In one report, mRNA levels were not altered by 7 days of haloperidol administered by continuous infusion (Morris et al. 1988). This study was different from ours in several respects, including route of drug treatment. As discussed below, route of administration may be an important factor (Gerfen et al. 1990). Striatal dynorphin immunoreactivity may be increased, reduced, or unchanged (Peterson and Robertson 1984; Quiron et al. 1985; Li et al. 1986; Nylander and Terenius 1986; Jiang et al. 1990) after treatment for 4–21 days. Treatment variables may again play a factor in the discrepancies. Taken together, the data suggest that acute VCMs are associated with reduced substance P and/or dynorphin mRNA shortly after the initiation of neuroleptic treatment and remain reduced for up to 3 weeks.

Striatal gene expression associated with acute VCMs is comparable to that seen in models of parkinsonism. For example, 6-hydroxydopamine treatment produces a hypokinetic state in rats and reduces both substance P and dynorphin mRNA (Young et al. 1986; Gerfen et al. 1990, 1991; Nisenbaum et al. 1994). Tardive VCMs, on the other hand, show changes similar to hyperkinetic

states. Although data linking the mRNA changes associated with VCMs directly to TD in humans is lacking, stimulant-induced behavioral activation in rats is associated with increased dynorphin and/or substance P mRNA (e.g. see Gerfen et al. 1990; Hurd and Herkenham 1992; Hurd et al. 1992; Daunais et al. 1993). These different patterns of mRNA expression suggest that the mechanisms involved in the generation of acute and tardive VCMs are different. Enkephalin mRNA levels are elevated in both types of VCMs. Similar elevations have been reported in the parkinsonian rat model as well as in hyperkinetic states, such as that induced by cocaine (Hurd et al. 1992) and thus may be involved in both types of movement disorders.

Differences between acute and tardive VCMs raise several issues. First, it is unclear when VCMs switch from being dystonic to dyskinetic in nature. Additional studies are needed to assess, for example, when VCMs become suppressible. From the current study, it is uncertain whether VCMs beyond 8 days can be suppressed. It seems likely that VCMs are not suppressible for at least 21 days, given the parkinsonian neurochemical changes and the lack of suppression with 0.4 mg/kg haloperidol. Second, the results would predict that, in humans, bolus administration would produce more EPS compared to depot treatment. It is unclear whether this is the case. Third, they raise questions about the role of acetylcholine. Differential response of acute and tardive VCMs to anticholinergic agents could provide important corroboration that they are distinct. Acute VCMs have been reported to decrease following treatment with anticholinergic agents. Paradoxically, suppression of tardive VCMs following anticholinergics has also been reported, but these results may be confounded. The behavioral activating effects of anticholinergics could reduce VCM counts through behavioral competition rather than true suppression (see Stoessl et al. 1989, for discussion). Further studies are needed to clarify the role of the cholinergic system in VCMs and oral behavior in rats.

It seems puzzling that VCMs are noted acutely in some studies but only after an extended delay in others. A number of variables could contribute to these inconsistencies, such as method of assessment, group size, dose, and route of administration (see See and Ellison 1990a; Egan et al. 1995b). Of studies that have used large groups and open cages for VCM counts, route of administration seems particularly important (See and Ellison 1990a). Most studies using decanoate injections find increased VCMs (3–10 times) only after prolonged treatment (Gunne et al. 1982; Gunne and Häggström 1983; Mithani et al. 1987; Stoessl et al. 1989; Egan et al. 1995a; Hyde et al. 1995; although see Jorgensen et al. 1994). In contrast, *rapid* onset of VCMs typically follows oral (Rupniak et al. 1983, 1985; Glenthoj and Hemmingsen 1989) or IP administration (Glassman and Glassman 1980; Rosengarten et al. 1983, 1986; Rupniak et al. 1986; Steinpreis et al. 1993). Oral treatments often yield lower counts (twice basal levels) during the first several weeks of treatment (Rupniak et al. 1983, 1985, 1986; Glenthoj and Hemmingsen 1989), but, with long term treatment, induce the same magnitude of VCM scores seen with decanoate injection (e.g. Rupniak et al. 1983; Waddington et al. 1983; Gunne et al. 1986; Johansson et al. 1986; Tamminga et al. 1990; Kaneda et al. 1992; although see See and Ellison 1990a). In contrast, acute IP injections produce high VCM counts shortly after administration (Rupniak et al. 1983; Steinpreis et al. 1993), as in the current study. In studies that have used both treatment methods, direct comparisons have shown that IP injections yield higher VCM scores than oral treatment (Glassman and Glassman 1980) which in turn induces higher rates than chronic infusion (See and Ellison (1990a,b).

Although data are limited, simple differences in plasma or brain neuroleptic levels do not appear to explain differences between acute and tardive VCMs. Preliminary data on a small group of rats (two or three per group) suggest that acute IP injections of 0.005, 0.05, 0.1, and 1.0 mg/kg haloperidol produce brain levels 1 h later of 3.6, 41.5, 78.7, and 556.7 ng/g, respectively. In contrast, rats with tardive VCMs that have been withdrawn from chronic haloperidol decanoate have brain levels of approximately 61.5 ng/g (Egan et al. 1994a), the equivalent of 0.05–0.1 mg/kg given acutely.

Chewing movements in rats have been reported following a variety of pharmacological manipulations, such as cholinergic agonists, and D_1 agonists or antagonists (Rosengarten et al. 1983, 1986, 1994; Johansson et al. 1987; Salamone et al. 1990; Egan et al. 1994b). Of particular relevance for the current study are reports that both striatal dopamine depletion (Jicha and Salamone 1991) and IP injections with D_1 agonists (Rosengarten et al. 1983) both produce VCMs. It appears that chewing in rats is a nonspecific behavior that can be induced by a variety of agents, including drugs that have opposing effects (see also Gunne et al. 1988). In humans, age, poor dentition, and institutionalization have been implicated in the pathogenesis of TD. While it is unclear whether analogous factors affect tardive VCMs, neuroleptics clearly play a role, since the control group did not develop significant VCMs. Acute VCMs may develop with treatment that rapidly blocks D_2 receptors, such as subcutaneous and IP injections. Tardive VCMs, in contrast, appear to develop gradually during chronic, continuous administration with decanoate preparations, implanted minipumps, and oral treatment, followed by prolonged withdrawal. In support of this formulation, it is interesting to note that striatal neurochemical changes depend on the method of drug administration. In particular, intermittent treatment may have more pronounced effects on the D_1-mediated striatal neuropeptide gene expression relative to continuous treatment (Gerfen et al. 1990).

In conclusion, despite similar appearances, acute and tardive VCMs appear to have distinct neurochemical substrates. While the rapid induction of VCMs in some studies has argued against the validity of the VCM syndrome as a model for TD, the current data suggest that acute and tardive VCMs are different. Differences in the timing of VCM expression may be related, in part, to the

route of administration. While the relevance of these distinct VCMs to acute and chronic movement disorders in humans is unclear, the existence of a distinct, late onset, hyperkinetic movement disorder induced by long term treatment with neuroleptics may provide a useful model to study the neurobiology of TD.

References

APA Task Force on Tardive Dyskinesia (1992) Tardive dyskinesia – a task force report of the American Psychiatric Association. APA Press, Washington DC

Bannon MJ, Lee JM, Giraud P, Young A, Affolter H-U, Bonner TI (1986) Dopamine antagonist haloperidol decreases substance P, substance K, and preprotachykinin mRNAs in rat striatonigral neurons. J Biol Chem 261: 6640–6642

Bannon, MJ, Elliot PJ, Bunney EB (1987) Striatal tachykinin biosynthesis: regulation of mRNA and peptide levels by dopamine agonists and antagonists. Mol Brain Res 3: 31–37

Civelli O, Douglass J, Goldstein A, Herbert E (1985) Sequence and expression of the rat prodynorphin gene. Proc Natl Acad Sci USA 82: 4291–4295

Crossman AE (1990) A hypothesis on the pathophysiological mechanisms that underlie levodopa- or dopamine agonist-induced dyskinesia in Parkinson's disease: implications for future strategies in treatment. Move Disord 5: 100–108

Cruz CJ, Beckstead RM (1988) Quantitative radioimmunocytochemical evidence that haloperidol and SCH 23390 induce opposite changes in substance P levels of rat substantia nigra. Brain Res 457: 29–43

Daunais JB, Roberts DCS, McGinty JF (1993) Cocaine self-administration incrases preprodynorphin, but not c-fos, mRNA in rat striatum. Neuroreport 4: 543–546

DeLong MR (1990) Primate models of movement disorders of basal ganglia origin. Trends Neurosci 13: 281–289

Egan MF, Hurd Y, Hyde TM, Weinberger DR, Wyatt RJ, Kleinman JE (1994a) Alterations in dopamine receptor and neuropeptide gene expression in striatonigral and striatopallidal neurons in rats with vacuous chewing movements induced by chronic treatment with haloperidol. Synapse 18: 178–189

Egan MF, Bachus SE, Hyde TM. Ferguson JN, Wyatt RJ, Kleinman JE (1994b) Effects of D_1 antagonist SCH23390 on vacuous chewing movements and striatal neuropeptide mRNA following long term haloperidol decanoate. Soc Neurosci Abstr 513: 12

Egan MF, Hyde TM, Kleinman JE, Wyatt RJ (1995a) Neuroleptic-induced vacuous chewing movements in rodents: incidence and effects of long-term increases in haloperidol dose. Psychopharmacology 117: 74–81

Egan MF, Ferguson J, Hyde TM (1995b) Effects of habituation of assessment on vacuous chewing movements in rats treated with long term haloperidol decanoate. Pharmacol Biochem Behav 53: 401–410

Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr, Sibley DR (1990) D_1 and D_2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250: 1429–1432

Gerfen CR, McGinty JF, Young WS (1991) Dopamine differentially regulates dynorphin, substance P, and enkephalin expression in striatal neurons: in situ hybridization histochemical analysis. J Neurosci 11[4]: 1016–1031

Glassman RB, Glassman HN (1980) Oral dyskinesia in brain-damaged rats withdrawn from a neuroleptic: implication for models of tardive dyskinesia. Psychopharmacology 69: 19–25

Glenthoj B, Hemmingsen R (1989) Intermittent neuroleptic treatment induces long lasting abnormal mouthing in the rat. Eur J Pharmacol 164: 393–396

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

Gunne LM, Growdon J, Glaeser B (1982) Oral dyskinesia in rats following brain lesions and neuroleptic drug administration. Psychopharmacology 77: 134–139

Gunne LM, Andersson U, Bondesson U, Johansson P (1986) Spontaneous chewing movements in rats during acute and chronic antipsychotic drug administration. Pharmacol Biochem Behav 25: 897–901

Gunne LM, Bachus SE, Gale K (1988) Oral movements induced by interference with nigral GABA neurotransmission: relationship to tardive dyskinesias. Psychopharmacology 100: 459–469

Hanson G, Alphs L, Pradhan S, Lovenberg W (1981) Response of striatonigral substance P systems to a dopamine receptor agonist and antagonist. Neuropharmacology 20: 541–548

Hong JS, Yang H-Y, T, Costa E (1978) Substance P content of substantia nigra after chronic treatment with antischizophrenic drugs. Neuropharmacology 17: 83–85

Hurd YL, Herkenham M (1992) Influence of a single injection of cocaine, amphetamine, or GBR 12909 on mRNA expression of striatal neuropeptides. Brain Res [Mol Brain Res] 16: 97–104

Hurd YL, Brown EE, Finlay JM, Fibiger HC, Gerfen CR (1992) Cocaine self-administration differentially alters mRNA expression of striatal peptides. Mol Brain Res 13: 165–170

Hyde TM, Egan MF, Wing LL, Wyatt RJ, Weinberger DR, Kleinman JE (1995) Persistent catalepsy predicts the development of dyskinesias in rats treated with chronic injections of haloperidol decanoate. Psychopharmacology 118: 142–149

Jeste DV, Lohr JB, Clark, K, Wyatt RJ (1988) Pharmacological treatments of tardive dyskinesia in the 1980s. J Clin Psychopharmacol 8: 38S-48S

Jiang H-K, McGinty JF, Hong JS (1990) Differential modulation of striatonigral dynorphin and enkephalin by dopamine receptor subtypes. Brain Res 507: 57–64

Jicha GA, Salamone JD (1991) Vacuous jaw movements and feeding deficits in rats with ventrolateral striatal dopamine depletion: possible relation to parkinsonian symptoms. J Neurosci 11[12]: 3822–3829

Johansson P, Casey DE, Gunne LM (1986) Dose-dependent increases in rat spontaneous chewing rates during long-term administration of haloperidol but not clozapine. Psychopharmacol Bull 22: 1017–1019

Johansson P, Levin E, Gunne LM, Ellison G (1987) Opposite effects of a D_1 and a D_2 agonist on oral movements in rats. Eur J Pharmacol 134: 83–88

Jorgensen HA, Andreassen OA, Hole K (1994) The relationship between motor effects in rats following acute and chronic haloperidol treatment. Psychopharmacology 116: 89–92

Kaneda H, Shirakawa O, Dale J, Goodman L, Bachus SE, Tamminga CA (1992) Co-administration of progabide inhibits haloperidol-induced oral dyskinesias in rats. Eur J Pharmacol 212: 43–49

Krause JE, Chirgwin JM, Carter MS, Xu ZS, Hershey AD (1987) Three rat preprotachykinin mRNAs encode the neuropeptides substance P and neurokinin A. Proc Natl Acad Sci USA 84: 881–885

Li S, Sivam SP, Hong JS (1986) Regulation of the concentration of dynorphin A(1–8) in the striatonigral pathway by the dopaminergic system. Brain Res 398: 390–392

Li SJ, Sivam SP, McGinty JF, Huang YS, Hong JS (1987) Dopaminergic regulation of tachykinin metabolism in the striatonigral pathway. J Pharmacol Exp Ther 243: 792–798

Mithani S, Atmadja S, Baimbridge KG, Fibiger HC (1987) Neuroleptic-induced oral dyskinesias: effects of progabide and lack of correlation with regional changes in glutamic acid decarboxylase and choline acetyltransferase activities. Psychopharmacology 93: 94–100

Morris BJ, Hollt V, Herz A (1988) Dopaminergic regulation of striatal proenkephalin mRNA and prodynorphin mRNA: contrasting effects of D_1 and D_2 antagonists. Neuroscience 25[2]: 525–532

Nisenbaum LK, Kitai ST, Gerfen CR (1994) Dopaminergic and muscarinic regulation of striatal enkephalin and substance P

messenger RNAs following striatal dopamine denervation: effects of systemic and central administration of quinpirole and scopalamine. Neuroscience 63: 435–449
Nylander I, Terenius L (1986) Chronic haloperidol and clozapine differentially affect dynorphin peptides and substance P in basal ganglia of the rat. Brain Res 380: 34–41
Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, 2nd edn. Academic Press, New York
Peterson MR, Robertson HA (1984) Effect of dopaminergic agents on levels of dynorphin (1–8) in rat striatum. Prog Neuro-Psychopharmacol Biol Psychiatry 8: 725–728
Pollack AE, Wooten GF (1992) D_2 dopaminergic regulation of striatal preproenkephalin mRNA levels is mediated at least in part through cholinergic interneurons. Mol Brain Res 13: 35–41
Quirion R, Gaudreau P, Martel J-C, St Pierre S, Zamir N (1985) Possible interactions between dynorphin and dopaminergic systems in rat basal ganglia and substantia nigra. Brain Res 331: 358–362
Radke JM, MacLennan AJ, Vincent SR, Fibiger HC (1988) Comparison between short- and long-term haloperidol administration on somatostatin and substance P concentrations in the rat brain. Brain Res 445: 55–60
Rosengarten H, Schweitzer JW, Friedhoff AJ (1983) Induction of oral dyskinesias in naive rats by D_1 stimulation. Life Sci 33: 2479–2482
Rosengarten H, Schweitzer JW, Friedhoff AJ (1986) Selective dopamine D-2 receptor reduction enhances a D-l mediated oral dyskinesia in rats. Life Sci 39: 29–35
Rosengarten H, Schweitzer JW, Friedhoff AJ (1994) Possible genetic factors underlying the pathophysiology of tardive dyskinesia. Pharmacol Biochem Behav 49(3): 663–667
Rupniak NMJ, Jenner P, Marsden CD (1983) Cholinergic manipulation of perioral behaviour induced by chronic neuroleptic administration to rats. Psychopharmacology 79: 226–230
Rupniak NMJ, Jenner P, Marsden CD (1985) Pharmacological characterisation of spontaneous or drug-associated purposeless chewing movements in rats. Psychopharmacology 85: 71–79
Rupniak NMJ, Jenner P, Marsden CD (1986) Acute dystonia induced by neuroleptic drugs. Psychopharmacology 88: 403–419
Salamone JD, Johnson CJ, McCullough LD, Steinpreis RE (1990) Lateral striatal cholinergic mechanisms involved in oral motor activities in the rat. Psychopharmacology 102: 529–534
See R, Ellison G (1990a) Intermittent and continuous haloperidol regimens produce different types of oral dyskinesias in rats. Psychopharmacology 100: 404–412
See R, Ellison G (1990b) Comparison of chronic administration of haloperidol and the atypical neuroleptics, clozapine and raclopride, in an animal model of tardive dyskinesia. Eur J Pharmacol 181: 175–186
Shibata K, Haverstick DM, Bannon MJ (1990) Tachykinin gene expression in rat limbic nuclei: modulation by dopamine antagonists. J Pharmacol Exp Ther 255: 388–392
Steinpreis RE, Baskin P, Salamone JD (1993) Vacuous jaw movements induced by sub-chronic administration of haloperidol: interactions with scopolamine. Psychopharmacology 111: 99–105
Stoessl AJ, Dourish CT, Iversen SD (1989) Chronic neuroleptic-induced mouth movements in the rat: suppression by CCK and selective dopamine D_1 and D_2 receptor antagonists. Psychopharmacology 98: 327–379
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
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
Waddington JL, Molloy AG (1987) The status of late-onset vacuous chewing/perioral movements during long-term neuroleptic treatment in rodents: tardive dyskinesia or dystonia? Psychopharmacology 91: 136–137
Waddington JL, Cross AJ, Gamble SJ, Bourne RC (1983) Spontaneous orofacial dyskinesia and dopaminergic function in rats after 6 months of neuroleptic treatment. Science 220: 530–532
Waddington JL, Youssef HA, O'Boyle KM, Molloy AG (1986a) A reappraisal of abnormal involuntary movements (tardive dyskinesia) in schizophrenia and other disorders: animal models and alternative hypotheses. In: Winlow W, Markstein T (eds) The neurobiology of dopamine systems. Manchester University Press, Manchester, pp 266–286
Waddington JL, Molloy AG, O'Boyle KM, Youssef HA (1986b) Spontaneous and drug-induced dyskinesias in rodents in relation to ageing and long-term neuroleptic treatment: relationship to tardive dyskinesia. In: Shagass C, Josiassen RC, Bridger W, Weiss K, Stoff D, Simpson G (eds) Biological psychiatry 1985. Elsevier, N.Y., pp 1151—1153
Yoshikawa K, Williams C, Sabol SL (1984) Rat brain preproenkephalin mRNA. J Biol Chem 259: 14301–14308
Young SW, Bonner TI, Brann MR (1986) Mesencephalic dopamine neurons regulate the expression of neuropeptide mRNAs in the rat forebrain. Proc Natl Acad Sci USA 83: 9827–9831