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Differential Effects of Within-Day Continuous *Vs* Transient Dopamine D_2 Receptor Occupancy in the Development of Vacuous Chewing Movements (VCMs) in Rats

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Accumulating evidence suggests that antipsychotics (APs) that lead to sustained blockade of dopamine D_2 receptors are more likely to induce acute extrapyramidal side effects (EPS) compared to APs that only occupy D_2 receptors transiently. It is unclear, however, whether a similar relationship exists for long-term AP-induced motoric side effects like tardive dyskinesia (TD). The objective of this study was to ascertain whether transient (via daily subcutaneous (s.c.) injections) *vs* continuous (via osmotic minipump) AP-induced D_2 receptor occupancy differentially affects the development of haloperidol-induced vacuous chewing movements (VCMs), an animal model of TD. Six groups of 12 rats received 0.1, 0.25, or 1 mg/kg of haloperidol or vehicle ($n = 36$) via osmotic minipump (to provide within-day sustained) or daily s.c. injection (within-day transient) for 8 weeks. VCMs were measured on a weekly basis and D_2 occupancy levels were measured *in vivo* using [^{3}H]-raclopride at the end of the experiment. Minipump-treated rats developed HAL dose-dependent D_2 occupancies of 0.1 mg/kg/day (57%), 0.25 mg/kg/day (70%), and 1 mg/kg/day (88%). S.C.-treated rats also developed HAL dose-dependent D_2 occupancies of 0.1 mg/kg/day (83% peak, 3% trough), 0.25 mg/kg/day (89% peak, 0% trough), and 1 mg/kg/day (94% peak, 17% trough). A total of 43% of rats given 0.25 and 1 mg/kg/day of HAL via minipump developed high VCMs compared to only 8% of the rats given the same doses via daily s.c. injections. The 0.1 mg/kg dose did not give rise to VCMs beyond vehicle levels regardless of the route of administration. These findings support the contention that D_2 occupancy levels induced by chronic HAL must be high *and* sustained through the day before significant risk of VCMs, and perhaps also TD, emerges.
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INTRODUCTION

Antipsychotic (AP) medications represent the mainstay of treatment in psychosis, and it is known that all currently available APs block dopamine D_2 receptors, regardless of their effects on other neurotransmitter systems (Kapur and Remington, 2001a). While D_2 blockade is associated with AP response, it is also responsible for several side effects, including extrapyramidal motor symptoms (presumably involving the nigrostriatal pathway).

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Using *in vivo* techniques, specifically positron emission tomography (PET), it has been possible to establish thresholds of D_2 blockade that can be linked to both clinical response and D_2-related adverse events in first-episode patients. It has been demonstrated that optimal chance of clinical response occurs with D_2 blockade exceeding 60–70% (Farde *et al*, 1992; Kapur *et al*, 1999, 2000a), whereas the risk of EPS increases substantially at levels of D_2 occupancy exceeding 80% (Remington and Kapur, 2000). Remarkably, this relationship is also observed in animal models of acute EPS (catalepsy) and clinical efficacy (conditioned avoidance response) after AP administration (Wadenberg *et al*, 2000, 2001).

Moreover, while it appears that a certain level of D_2 receptor occupancy must be reached for clinical responses to occur, recent evidence suggests that such target occupancy levels need not be continually maintained, that is, that AP effects can be achieved without sustained D_2 occupancy (Carpenter *et al*, 2000; Kapur and Seeman, 2001;

Seeman, 2002). Recent PET studies in humans have shown that clinically effective doses of APs such as clozapine or quetiapine produce high levels of D_2 occupancy ($>60\%$) shortly after drug administration, but very low levels when occupancy was measured 12 h after drug ingestion (Kapur *et al*, 2000b). Thus, it appears that these occupancy levels do not need to be continuous for therapeutic effects to occur.

This raises the question as to whether these same benefits related to a lack of continuous D_2 occupancy might also carry over to long-term side effects which have been linked to prolonged D_2 blockade, specifically tardive dyskinesia (TD), a late-occurring motor side effect linked to acute EPS (Casey and Keepers, 1988). To this end, we recently found that only haloperidol administered through tri-weekly decanoate injections, but not daily intraperitoneal (i.p.) injections, led to high levels of VCMs (Turrone *et al*, 2002). These observations, coupled with evidence demonstrating that acute subcutaneous (s.c.) injections of haloperidol leads to only transiently high levels of D_2 occupancy (Kapur *et al*, 2000c), raised the hypothesis that transient D_2 occupancy might also not induce VCMs and TD.

To this end, we have chosen to study vacuous chewing movement (VCM) in rats, an animal model that shares substantive features with human TD (for a review see Turrone *et al*, 2002). VCMs are characterized by purposeless (nondirected) mouth openings in the vertical plane, with or without tongue protrusion (eg Iversen *et al*, 1980). VCMs have been shown to develop secondary to various pharmacological (eg dopamine depletion, cholinomimetics) and surgical lesions (Salamone *et al*, 1998). Most notably, there are over 75 studies that have studied the use of APs in this model; the vast majority have found that the chronic administration of typical APs can precipitate the onset of VCMs, and more recently, that several atypicals such as clozapine and olanzapine do not (Gao *et al*, 1998; Steinpreis *et al*, 1997). Moreover, there is marked individual variability in the number of rats who develop the syndrome; only 45–55% of all treated rats develop high levels of VCMs despite being exposed to high doses of the same AP (Hashimoto *et al*, 1998). There also appears to be an effect of age on VCM development; older rats have been found to have higher baseline VCMs compared to their younger counterparts (Steinpreis *et al*, 1997). Furthermore, VCMs may increase and/or persist for several weeks after drug withdrawal and can also be suppressed with an acute AP challenge (Glenthoj, 1993).

The goal of the present investigation was therefore to address the question of whether the risk of VCMs diminishes with transient *vs* continuous D_2 occupancy. Using haloperidol as a prototypical AP, we hypothesized that continuous receptor occupancy achieved by delivery through osmotic minipumps would result in higher levels of VCMs than the same dose delivered through daily s.c. injections.

METHODS

Animals

Adult male Sprague–Dawley rats (Charles River, Montreal, Quebec), weighing 175–200 g at the start of the experiment, were housed two per cage in $19 \times 101/2 \times 8$ transparent polycarbonate cages (Lab Products Inc., Seaforth, DE, USA) and maintained on a 12-h light/dark cycle with continuous access to food and water. All procedures were carried out in accordance with The Animals for Research Act 1968–1969 of the Province of Ontario and the Guidelines of the Canadian Council on Animal Care.

Drug Treatments

Groups of 12 rats each were randomly assigned to receive one of the following treatments: 0.1, 0.25, or 1 mg/kg/day of haloperidol (McNeil Pharmaceuticals, Spring House, PA) via Alzet osmotic minipumps (Alzet model 2ML4, Durect Corporation, Cupertino, CA) + daily vehicle s.c. injections, vehicle via osmotic minipumps + 0.1, 0.25, or 1 mg/kg haloperidol via daily s.c. injections, or vehicle via minipumps + vehicle via daily s.c. injections for a total of 8 weeks. Haloperidol doses were calculated based on weights derived from previous animal studies; 350 g average (for beginning of week 3) for the first pump and 430 g average (for beginning of week 6) for the second and final pump. This was carried out to ensure that all rats were given the same drug concentration.

Osmotic Minipump Surgery

After 1 week of habituation, each rat was anesthetized using the inhalant anesthetic isoflourane. Once sedated, a portion of the animal's back was shaved and sterilized with both isopropyl alcohol and betadine solution. A 1/2-in incision was made on the back and blunt-tipped scissors were used to loosen connective tissue. The minipumps were chemically cleaned with isopropyl alcohol and then inserted according to the manufacturer's specifications. The incision was closed using a combination of both Vetbond glue and surgical staples to ensure proper closure. Postoperative animals were then placed in a heated plexiglass cage until the anesthetic wore off (approximately 4 min). After 28 days, animals were re-anesthetized and a second incision was made in close proximity to the first. Old pumps were removed and new pumps were inserted containing the same drug solution, although the dose was adjusted to compensate for the increase in body weight. All other procedures were repeated as previously described.

Assessment of VCMs

Individual rats were observed on a flat round surface (26 cm diameter, 50 cm high) on a weekly basis (23 h after the previous s.c. injection) by a trained rater who was blind to treatment. Following a 2-min acclimatization period, the number of VCMs was counted during a 2-min test period. When chewing movements were sequential, the number of individual jaw movements was counted. Oral movements were counted only if they appeared to be purposeless, that is, not directed at specific objects. The inter-rater reliability is 0.84 in our laboratory, as calculated after VCM counts performed by two investigators were compared during a previous experiment. For additional analyses, VCM behavior was dichotomized as 'High' (+) *vs* 'Low' (−), with rats scoring $\geqslant 8$ VCMs averaged over the last three sessions

being rated as VCM(+) and those with mean scores <8 as VCM(−), following a commonly used criterion in the literature (Egan *et al*, 1996; Sasaki *et al*, 1996). In these analyses, VCM(+) rats are referred to as showing the VCM syndrome.

D_2 Receptor Binding Potential and Occupancy

After 8 weeks, animals were randomly selected from each group and killed for determination of either peak (2 h post-HAL) or trough (24 h post-HAL) D_2 occupancies. Rats were administered [^{3}H]-raclopride (7.5 μCi/rat in a volume of 0.4 ml 0.9% NaCI solution) injected into the tail vein 30 min before being killed. The animals were decapitated and the striatum and cerebellum were immediately dissected. The cerebellum was homogenized with a small spatula, and 50–100 mg was collected in previously weighed 20 ml glass scintillation vials. The vials were then weighed with tissue, and 2 ml Solvable (Canberra Packard, Canada) was added to the vials. The vials were kept on an automated shaking tray, and gently agitated for 24 h at 23°C. Thereafter, 5 ml of Aquasure fluid was added, and the mixture was allowed to shake for another 24 h. Quantitation of [^{3}H]-raclopride radioactivity was determined by liquid scintillation spectrometry using the Beckman LS5000 CE liquid scintillation counting system at 50% efficiency. Striatal and cerebellar counts were obtained and expressed as disintegrations per min/mg (DPM/mg).

The D_2 receptor binding potential was defined as striatum-cerebellum/cerebellum. The value for the control group was pooled, and occupancy in each animal was then determined using the same formula as used in human studies (Kapur *et al*, 1999, 2000a):

$$\%\text{Occupancy} = 100 \times (D_2BP_{control} - D_2BP_{indiv}/D_2BP_{control})$$

Haloperidol Plasma Levels

Blood samples were obtained for each animal at the same time they were killed for D_2 occupancy determination. Haloperidol levels were obtained according to the methods described by Tomlinson *et al* (1995). Briefly, haloperidol levels were measured through a liquid–liquid extraction followed by a liquid chromatography-mass spectrometry analysis conducted with an HP 1100 LC-DAD-MSD system (Hewlett-Packard Company, USA), using an electrospray source and controlled by HP LC-MSD Chemstation software (St Joseph's Health Centre, Ontario). This method has a lower limit of detection of 0.5 nmol/l, a lower level of quantification of 1 nmol/l, and a linearity limit of 212 nmol/l.

Data Analysis

The primary dependent variables for the studies were AP dose, VCMs, D_2 receptor occupancy, plasma levels, and route of administration. Data are presented as the mean (± SEM) for all rats in each group. Data were analyzed using repeated measures analysis of variance (ANOVA) followed by Bonferroni *post hoc* analyses when ANOVA results were significant. Spearman correlations were calculated to determine the strength of the relationship between each of the aforementioned variables.

RESULTS

D_2 Receptor Occupancy: Daily S.C. Injections

Different doses of haloperidol s.c. injections at peak (2 h postinjection) gave rise to mean D_2 occupancies of 83 ± 4% (0.1 mg/kg/day), 89 ± 1% (0.25 mg/kg/day), and 94 ± 3% (1 mg/kg/day). D_2 occupancies at trough (24 h postinjection) declined to 3 ± 5% (0.1 mg/kg/day), 0 ± 0% (0.25 mg/kg/day), and 17 ± 7% (1 mg/kg/day). Mean peak D_2 occupancies for the s.c. groups were not significantly different between doses, whereas trough D_2 occupancies were ($P<0.0001$).

D_2 Receptor Occupancy: Osmotic Minipump

In order to maintain consistency, minipump rats were also subdivided into two groups for peak and trough D_2 determination, although the mean occupancy values were not significantly different for the latter group between sample times. Rats administered haloperidol through minipumps achieved mean D_2 occupancies of 57 ± 4% (0.1 mg/kg/day), 70 ± 3% (0.25 mg/kg/day), and 88 ± 4% (1 mg/kg/day). Mean D_2 occupancies were significantly different between different haloperidol doses administered through osmotic minipump ($F(3,67) = 419.50$ $P<0.0001$). Bonferroni-adjusted pairwise comparisons revealed that mean D_2 occupancies were significantly different between all dose pairs ($P<0.01$).

VCM Behavior: Daily S.C. Injections

An ANOVA comparing mean VCMs between different doses of haloperidol administered via daily s.c. injections over the last three test sessions (Table 1) failed to reveal any significant differences between doses ($F(3,67) = 2.1$, $P = 0.11$). VCM scores during the treatment period are shown in Figure 1. A repeated measures ANOVA comparing VCMs revealed a nonsignificant dose effect ($F(3,67) = 1.94$, $P = 0.13$), a significant time effect ($F(8,536) = 4.46$, $P<0.001$), and a nonsignificant dose × time effect

Table 1 VCM Scores[a], Haloperidol Plasma Levels, and the D_2 Receptor Occupancy

Haloperidol daily dose (mg/kg)	0.00	0.1	0.25	1
Minipump groups				
VCM score	2 ± 1	2 ± 1	8 ± 2	7 ± 2
D_2 occupancy (%)	0 ± 0	57 ± 4	70 ± 3	88 ± 4
Plasma level (nmol/l)	0 ± 0	4 ± 1	8 ± 1	14 ± 4
Daily s.c. injection groups				
VCM score	2 ± 1	1 ± 1	1 ± 1	3 ± 1
Peak (2 h) D_2 occupancy	0 ± 0	83 ± 4	89 ± 1	94 ± 3
Trough (24 h) D_2 occupancy	0 ± 0	3 ± 5	0 ± 0	17 ± 7
Peak plasma level (nmol/l)	0 ± 0	5 ± 1	24 ± 2	94 ± 7
Trough plasma level (nmol/l)	0 ± 0	ND[b]	ND[b]	1 ± 1

[a]Mean VCM scores over the last three test sessions ± SEM.
[b]ND = nondetectable.

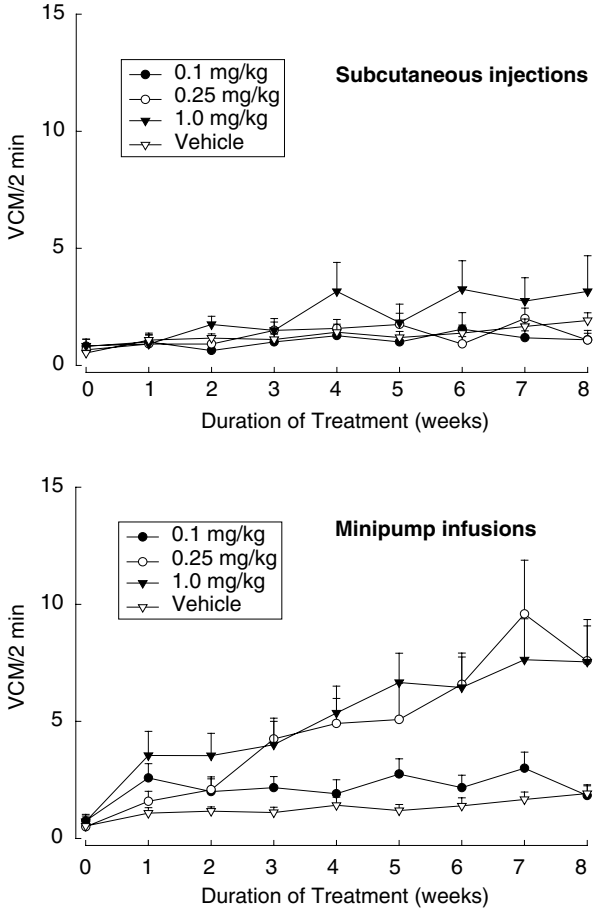

Figure 1 Number of VCMs in rats receiving 0.1, 0.25, or 1 mg/kg/day of haloperidol or vehicle administered either via daily s.c. injections (top panel) or via osmotic minipumps (bottom panel). Each point represents a mean ($\pm$ SEM) of eight animals per group. VCMs averaged over the last 3 weeks were not significantly different between doses ($P = 0.11$) in s.c.-treated groups, but were significantly different in minipump-treated groups (bottom panel, $F(3,67) = 18.61$, $P < 0.0001$).

($F(24,536) = 1.13$, $P = 0.31$). When VCM behavior was dichotomized as VCM(+) *vs* VCM(−), 17% (2/12) of rats in the 1 mg/kg daily s.c. group were classified as VCM(+) compared to 0% in the 0.25 mg/kg group, 0% of the 0.1 mg/kg group, and 0% in the vehicle group. Haloperidol plasma levels are reported in Table 1. As expected, haloperidol plasma levels for each dose administered through daily s.c. injections were high at peak (2 h postinjection) and significantly lower when measured at trough (24 h postinjection).

VCM Behavior: Osmotic Minipump

All groups were similar with respect to predrug baseline VCMs ($F(6,99) = 0.30$, $P = 0.88$). An ANOVA comparing mean VCMs between different doses of haloperidol administered via osmotic minipump over the last three test sessions (Table 1) revealed significant differences among doses ($F(3,67) = 18.61$, $P < 0.0001$). Rats administered 0.25 and 1 mg/kg/day had significantly ($P < 0.002$) higher VCMs compared to 0.1 and vehicle groups, although they were not significantly ($P > 0.50$) different from each other. VCM scores during the treatment period are shown in Figure 1. A repeated measures ANOVA revealed that there was a significant dose effect ($F(3,67) = 22.08$, $P < 0.0001$), time effect ($F(8,536) = 22.75$, $P < 0.0001$), and dose × time effect ($F(24,536) = 5.43$, $P < 0.0001$). Bonferroni *post hoc* analyses for dose revealed significantly higher VCMs in the 1 mg/kg haloperidol compared to 0.1 mg/kg HAL-treated rats ($P = 0.01$) and vehicle ($P < 0.001$), but not compared to the 0.25 mg/kg group ($P = 0.10$). The 0.25 mg/kg group also demonstrated significantly higher VCMs than the vehicle-treated ($P < 0.001$) and 0.1 mg/kg ($P = 0.002$) groups. When VCM behavior was dichotomized as VCM(+) or VCM(−), 45% (5/11) of rats in the 1 mg/kg minipump group were classified as VCM(+) compared to 33% in the 0.25 mg/kg group, 0% in the 0.1 mg/kg group, and 0% in the vehicle group (Figure 2). The 0.25 and 1 mg/kg/day groups were not significantly different ($P > 0.50$, Fisher's exact test).

Table 2 Spearman Correlations for Minipump Rats

	Dose	Plasma level	D_2 occupancy
Dose			
Plasma level	0.79*		
D_2 occupancy	0.76*	0.69*	
VCM score	0.49*	0.46*	0.40**

Bonferroni-adjusted probabilities *$P < 0.001$, **$P = 0.02$.

Haloperidol plasma levels are reported in Table 1. As expected, haloperidol plasma levels for each dose administered through minipump were lower than that obtained by administering the same dose of the haloperidol via s.c. injection when measured at peak (2 h postinjection), but higher than that measured at trough (24 h postinjection). Spearman correlations between plasma levels and the other dependent variables are shown in Table 2.

Mode of D_2 Occupancy Effect

In all, 43% (10/23) of rats given 0.25 or 1 mg/kg/day doses of haloperidol via minipump (achieving mean D_2 occupancy of 82%) developed high (beyond the eight VCM/2 min cutoff as defined by Egan *et al*, 1996) levels of VCMs (13 VCMs/2 min) compared to only 8% (2/24) of the rats given the same doses via daily s.c. injections, despite the fact that they also yielded high (>85%), albeit transient, mean D_2 occupancy levels. The 0.1 mg/kg dose did not give rise to any VCMs beyond vehicle, regardless of the route of administration. Moreover, when the number of VCM(+) animals were categorized according to the route of administration, it was found that VCM(+) rats were significantly more likely to have received haloperidol via minipump (84%) as opposed to daily s.c. injections (17%), irrespective of the haloperidol dose administered ($P = 0.02$, Fisher's exact test).

DISCUSSION

The results of the present study demonstrate a clear association between the emergence of the VCM syndrome and continuous *vs* transient D_2 receptor occupancy achieved by different regimens of drug administration. Animals who received haloperidol via osmotic minipumps

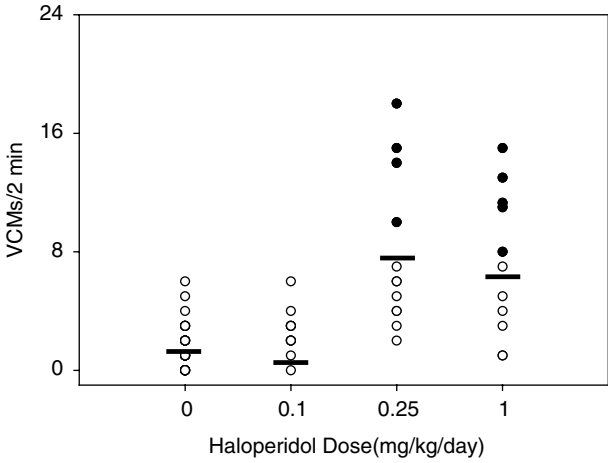

Figure 2 Mean (final 3 weeks) VCMs per individual rat treated with different doses of haloperidol or vehicle via minipumps. Filled circles indicate VCM(+) rats.

(giving rise to *continuous* moderately high D_2 occupancy) developed significantly higher levels of VCMs compared to those receiving the same doses of haloperidol via daily s.c. injections (giving rise to *transiently* high levels of D_2 occupancy).

These data help clarify the results derived from previous studies (Andreassen and Jorgensen, 1994, Turrone *et al*, 2002). We recently conducted an 8-week study involving daily i.p. injections of haloperidol that failed to demonstrate either a significant increase in VCMs *vs* placebo or a clear dose–response relationship (Turrone *et al*, 2002). Another study involving daily administration of haloperidol via the intracerebroventricular (i.c.v.) route, a technique analogous to intermittent daily dosing, also was unable to increase VCMs reliably beyond a certain threshold (Andreassen and Jorgensen, 1994). In fact, almost all investigations using haloperidol and reporting high rates of VCMs administered the drug through long-acting decanoate injections or drinking water, approaches leading to continuous D_2 occupancy. Taken together, these data suggest that transient D_2 occupancy is significantly less likely to induce the VCM syndrome.

This novel finding parallels other research conducted by Volkow *et al* (2002) in drug addiction, providing further evidence for the differential effects of dopamine brain kinetics on behavioral responses. Both cocaine and methylphenidate have been found to block similar degrees of dopamine transporter (DAT) in the striatum using PET imaging. Moreover, they found that the faster the DAT blockade, the faster the increase in dopamine release and postsynaptic DA receptor activation. Despite the fact that both drugs block DAT, however, methylphenidate appears to have a reduced abuse potential due to its slower clearance rate from the brain. Conversely, cocaine has a high potential of abuse since it has a much faster clearance rate from the brain, leading to an increased frequency of drug self-administration. Thus, the time course of neurotransmitter modulation can clearly lead to very different effects on behavior.

In addition, a dose-dependent effect was found only in the group exposed to sustained blockade, with syndrome-defining levels (>8 VCMs/2 min) beginning to emerge at the 0.25 mg/kg/day haloperidol dose, a finding reported in other studies using haloperidol in the VCM model (Turrone *et al*, 2002, 2003). Moreover, there is also some evidence that AP dose is a risk factor for TD, with higher doses increasing the likelihood that a patient will develop TD (Kane *et al*, 1986). While this relationship is complicated due to methodological issues (eg widely varying plasma levels despite equal AP dose), Kane found that the risk of TD dropped two-fold with a 10-fold lower dose in a long-term study using depot fluphenazine decanoate.

It is necessary to note that past clinical studies evaluating the potential benefits of 'intermittent' AP dosing actually reported an increased risk of TD (Kane *et al*, 1986, Van Harten *et al*, 1998). However, it should be pointed out that the 'intermittent' clinical approach is very different from the 'transient' occupancy used here. In this case, the animals received drug every day—the only issue being whether occupancy was or was not allowed to decline to very low trough levels every 24 h. In contrast, the intermittent clinical approach involves having patients temporarily withhold taking their medication for several weeks or months. Therefore, data from intermittent clinical trials do not necessarily contradict the present findings.

The mechanisms through which continuous D_2 binding leads to significant increases in VCMs in susceptible rats remain unresolved and are likely to be complex. One possibility is that high levels of chronic sustained D_2 occupancy can lead to continuous increases in both dopamine and its metabolites (See and Kalivas, 1996), whereas transient occupancy avoids this effect. For example, extracellular dopamine and its metabolites have been found to be elevated in the striatum of animals undergoing chronic high-dose haloperidol treatment when administered through continuous release silastic reservoirs (See, 1993). This response can be problematic since data derived from both *in vitro* and *in vivo* studies suggest that these increases can generate high levels of oxidative stress that could eventually damage striatal neurons (Cadet and Lohr, 1989).

Secondly, haloperidol has also been found to generate both neurotoxic metabolites (Bloomquist *et al*, 1994; Galili *et al*, 2000) and reactive oxygen species (Sagara, 1998), effects that may be attenuated by the cotreatment of antioxidants such as vitamin E (Andreassen and Jorgensen, 2000). Our data demonstrate that minipump-treated animals had stable haloperidol plasma levels throughout the day, whereas subcutaneously treated rats experienced a dramatic decline over the same time period. It is possible that the latter group cleared the drug too quickly to allow for the accumulation of haloperidol, and thereby avoiding its neurotoxic effects.

A third potential mechanism relates to the concept of pharmacological tolerance *vs* sensitization. It has been theorized that APs administered in an intermittent mode render the dopaminergic motor system more sensitive to acute pharmacological manipulation and this has been suggested to explain the induction of motor side effects (Glenthoj *et al*, 1990). This has been supported by studies that have reported high levels of increased oral movements both during and after intermittent drug treatment (Glenthoj *et al*, 1990). However, both Kashihara *et al* (1986) and Ericson *et al* (1996) found that animals who received APs through osmotic minipumps developed a greater tolerance

response to DA turnover after a single injection of AP compared to animals who received the drug through daily injections. Moreover, the former study also found that only the continually treated animals showed an increase in D_2 receptors (measured by [^{3}H]-spiperone binding). In light of our present findings, it appears that DA turnover tolerance will be significantly lower in rats receiving AP medications through daily injections, since this method only leads to a very brief period of D_2 receptor occupancy. While we did not perform any acute challenge experiments in this study, it remains possible that our minipump-treated animals may have also developed greater dopamine-related tolerance, which would be in direct contrast to the contention that sensitization underlies AP-induced motor side effects.

An issue that could be raised regarding this study is the shorter (8 weeks) duration of treatment compared to other VCM investigations that have been longer than 6 months (eg Egan *et al*, 1996). While our study was limited to 8 weeks, the decision to reduce the length of the study was based on the finding that the VCM behavior plateaus beyond 5 weeks of treatment in this study and in other VCM experiments conducted in our laboratory (Turrone *et al*, 2002, 2003). In addition, there are other groups that utilize this model for an even shorter time period (3 weeks) within the context of TD research (Naidu *et al*, 2001). Furthermore, it is important to note that TD is diagnosed in humans after 3 months of continuous AP exposure (APA, 1994). In light of the fact that the average lifespan of the rat is 2 years compared to 75+ years in humans, it is not unreasonable to suggest that 2 months in the former, about a tenth of the lifespan, is likely to represent a longer episode in humans.

One other issue which warrants comment relates to the finding that rats develop high levels of VCMs within the first few weeks of treatment. This has led some to suggest that VCMs may be a better proxy of acute extrapyramidal side effects (EPS) (Rupniak *et al*, 1985) or Parkinsonian tremor (Salamone *et al*, 1998). Importantly, however, the evidence in support of these hypotheses is largely derived from studies where animals were treated with APs over a brief time period (<21 days) (see Turrone *et al*, 2002). There is some evidence that early-appearing VCMs may be more neurochemically and pharmacologically related to acute EPS than TD (Egan *et al*, 1996). Moreover, evidence derived from a recent long-term VCM study has demonstrated that it is not the anticholinergic challenge that reduces VCMs, but some aspect inherent to the injection procedure itself (Sakai *et al*, 2001). Thus, while the VCM model is not an exact replica of the human condition, there is a substantial body of evidence cited from multiple independent sources that provide sufficient support for its use as a proxy for TD.

In conclusion, the results of the present study strongly suggest that continuous D_2 occupancy (achieved via osmotic minipump) is important for the emergence of the VCM syndrome, whereas transient, albeit high (>85%) D_2 occupancy, is significantly less likely to induce VCM behavior. Moreover, a dose-dependent increase in VCMs can be demonstrated, but only in the group exposed to sustained D_2 occupancy. It does appear that other factors not examined in this study must also play a role, since 57% of the 0.25 and 1 mg/kg/day HAL pump-treated rats did not develop VCMs. This highlights the complexity of the VCM syndrome and possibly also TD, and the fact that our understanding of their precise pathophysiology remains limited.

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