Antipsychotic Tolerance: Evaluating the Behavioural Effects of Continuous Versus Intermittent Haloperidol Exposure.

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science

Institute of Medical Science
University of Toronto

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Abstract

There are several lines of investigation, both preclinical and clinical, that suggest antipsychotic drug (APD) tolerance may occur. Using a behavioural animal model, the present study examined whether the amount of APD exposure (i.e. continuous versus intermittent) significantly impacts APD efficacy. More specifically, it was investigated whether continuous haloperidol (an APD) exposure, in comparison to intermittent, would lead to a decrease in response over time. Catalepsy, locomotor activity, and exploratory behaviour were assessed weekly, over the course of 21 days, in rats receiving haloperidol continuously or intermittently. Our findings are consistent with earlier preclinical reports that have implicated chronic and continuous APD therapy in reduced drug effect. This line of investigation has immediate and direct implications in terms of current guidelines regarding maintenance antipsychotic treatment.
Moving forward, we are in a position to translate these results to clinical studies that can further examine this issue in humans.
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Steve Mann (Centre for Addiction and Mental Health) assisted in the setup of locomotion tracking equipment.

Dr. Patrick McCormick (Centre for Addiction and Mental Health) aided in the collection of plasma samples and provided valuable feedback regarding the experimental design.

Roger Raymond (Centre for Addiction and Mental Health) provided technical and procedural training on drug preparation and its delivery method.

Celine Teo (Centre for Addiction and Mental Health) aided in the collection of plasma samples.

Virginia Wilson (Centre for Addiction and Mental Health) provided procedural training on injections and osmotic mini-pump surgery.
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Introduction:

Schizophrenia

The term “schizophrenia” is just over a century old and comes from Greek origins, with *schizo phren* translating to “split mind” in English (Ashok et al. 2012). Though schizophrenia was not identified as its own illness until the late 1800s, it is believed to have roots dating back several thousands of years (Ashok et al. 2012).

Currently, epidemiological studies show that schizophrenia, one of psychiatry’s most debilitating mental illnesses, afflicts approximately 0.5-1.0% of the entire population (McGrath et al. 2004). It is seen in all cultures and ethnicities, and is equally prevalent in men and women.

Symptoms of schizophrenia typically begin during late adolescence, with the devastating nature of the illness compounded by its onset during such an important stage of development. Schizophrenia’s symptoms often wax and wane over the course of the illness, adversely impacting functional recovery; schizophrenia is associated with unemployment rates in the range of 80-90%, and affected individuals often are unable to return to their premorbid level of functioning (Leucht et al. 2012). In this regard, the illness also has a profound impact on families and caregivers, as well as at a societal level where its costs can be measured both directly and indirectly. For example, figures specific to
Canada for 2004 estimate the number of persons with schizophrenia at 234 305 (95% CI, 136 201-333 402). Direct healthcare and non-healthcare costs were calculated to be 2.02 billion CAN dollars, with the high unemployment rate and lost productivity adding indirect costs of 4.83 billion CAN dollars out of 6.85 billion CAN dollars. Notably, the greatest proportion of total costs is reflected in productivity losses i.e. 70% of total costs (Goeree et al. 2005).

**Schizophrenia: Symptoms and Diagnosis**

No symptoms are pathognomonic for schizophrenia, as they can be seen in all conditions characterized by psychosis. Historically, and not surprisingly given the nature of psychosis (e.g. hallucinations, delusions), the focus has been on what are termed the “positive” symptoms, referred to as such because they can be conceptualized as an excess in an individual's normal functioning. Disturbances in perception frequently accompany positive symptoms, such as auditory hallucinations that present as voices commanding the individuals and/or commenting on them (Meyer et al. 2005). On the opposite end of the spectrum are “negative” symptoms, which represent a reduction or loss of functioning. Examples of negative symptoms include motivational impairment (avolition and/or amotivation) and affective blunting (i.e. absent or inappropriate emotion) (van Os et al. 2009). These symptoms are seen as playing a critical role in the marked functional impairment that characterizes so many individuals with this illness (Meyer et al. 2005). In addition, schizophrenia is associated with
neurocognitive impairments, for example in areas such as attention and working memory (van Os et al. 2009). In line with this, the illness is associated with various neurological features that include soft signs and even motor movements, although in the case of the latter it is difficult to disentangle those specific to the illness per se and movements associated with the long-term use of antipsychotic medications. From the standpoint of diagnosis, schizophrenia is defined in the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) as “a disturbance that lasts for at least 6 months and includes at least a month of active-phase symptoms” (Table 1).

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**Table 1.** Diagnostic criteria for Schizophrenia (DSM-IV-TR, American Psychiatric Association, 2000).
As noted, no specific symptoms are pathognomonic for schizophrenia, and in classification systems such as DSM-IV, numerous diagnostic categories share in common the possible presence of psychotic features (van Os et al. 2009). In order to differentiate between these disorders, clinicians must take into account symptom duration, course of illness, and potential diagnostic confounds (e.g. substance abuse, and the total constellation of symptoms e.g. depressive and/or manic features) that may guide decision-making regarding diagnosis (van Os et al. 2009).

It is important to acknowledge that risk of schizophrenia is, at least in part, genetic (Mulle 2012). Unfortunately, the precise nature of this relationship is less than clear, as reflected by the fact that even now there are no established genetic biomarkers that are used routinely in clinical practice to aid in diagnosis. Further, it is known that illnesses such as schizophrenia and bipolar disorder do not ‘breed true’; that is, considerable overlap can be seen between diagnostic categories across generations. At present, a family history of schizophrenia can at best simply be viewed as one of a number of potential risk factors.

**Antipsychotic Drugs**

Prior to the discovery of chlorpromazine, various treatments for schizophrenia had been introduced, but with less than compelling evidence supporting their ongoing use; procedures such as prefrontal lobotomy, insulin-induced seizures,
and electroconvulsive therapy (ECT) represent examples (Meyer et al. 2005). With the introduction of psychopharmacology in the 1950s, antipsychotic drugs (APDs), known first as “major tranquilizers” before being designated as “neuroleptics” (literally meaning ‘to take the neuron’), became the cornerstone of psychiatric treatment (Healy 2002). Like many medical advances, the discovery of APDs was, at least initially, unintentional. Chlorpromazine, a drug originally intended to enhance surgical anesthesia because of its anxiolytic properties, was unexpectedly found to therapeutically benefit patients experiencing psychotic symptoms (Healy 2002).

Over the next several decades, the actions of this class of drugs on dopamine, a neurotransmitter found within the brain, was linked to their antipsychotic efficacy, setting the stage for theories that implicated dopamine’s role in psychosis and, specifically, schizophrenia (Healy 2002).

The Dopamine Hypothesis of Schizophrenia

The earliest work examining the action of antipsychotics focused on norepinephrine (NE), another neurotransmitter within the central nervous system (CNS), although this soon gave way to the investigation of dopamine. The effect of neuroleptics on dopamine metabolism and in response dopamine agonists such as amphetamine provided the signal needed to implicate its role in the action of drugs like chlorpromazine (Carlsson 1959).
This would ultimately provide the framework for the theory of schizophrenia as a disorder of hyperdopaminergic activity, a model that continues to hold a central role even today, albeit in a more complex form that sees dopamine’s role as varied as a function of brain region and symptom domain. It was in the 1970s that the dopamine receptor was discovered and subtyped into D₁ and D₂ receptor subfamilies. This proved a critical development from the standpoint of explaining the action of neuroleptics as a relationship between D₂ affinity, and therapeutic response was established shortly thereafter. Figure 1. on the following page details how neuroleptics like chlorpromazine effect their antipsychotic activity through blockade of the D₂ receptor (Kapur et al. 2006).

**Figure 1.** Chlorpromazine’s inhibitory action on dopamine (www.mikeclaffey.com)
With this information in hand, attention turned to developing a new group of neuroleptics characterized by much greater affinity for the D<sub>2</sub> receptor, based on the notion that such a drug would represent the ‘ideal’ antipsychotic (keeping in mind that the model at that time was focused entirely on dopamine). Neuroleptics like haloperidol and pimozide represented this new group of drugs, which came to be designated “high potency” versus “low potency” agents, with drugs like chlorpromazine and thioridazine examples of the latter.

Clinically, these high-potency neuroleptics required much lower doses for efficacy, although this was countered by their more benign side effect profile in terms of cardiovascular (CVS) side effects (e.g. hypotension) linked to the more heterogeneous receptor binding profile of the low-potency neuroleptics. This permitted these drugs to be used at higher doses without the same restrictions invoked by CVS side effects, which resulted in several decades of mega-dosing with antipsychotics, reflected in treatment strategies such as “rapid neuroleptization” and the notion essentially that ‘more is better’ when trying to establish treatment response. Furthermore, it was emerging evidence in the 1970s that established the effects of APDs on the binding of haloperidol (Seeman et al. 1976). Specifically, it was reported that all clinically effective APDs (available at that time) block the stereospecific binding of <sup>3</sup>H-haloperidol at concentrations which correlate directly with the clinical potencies (Seeman et al. 1976).
As an aside, the prominent dopamine-binding affinities of the high-potency agents also came at a cost in terms of side effects. Blockade of dopamine at the level of the nigrostriatal dopaminergic pathway is associated with parkinsonian side effects (e.g. bradykinesia, tremor), while blockade at the level of the tuberoinfundibular dopaminergic pathway gives rise to hyperprolactinemia. Both of these side effects became routine in clinical practice as the high-potency drugs supplanted their low-potency counterparts, and were used at increasingly higher doses (Healy 2002). Part of the problem contributing to this practice was how antipsychotic dosing was established. Historically, it was based on peripheral pharmacokinetics and the terminal half-life ($t_{1/2}$) which, as would be discovered later, is not representative of CNS kinetics, at least in terms of the $D_2$ occupancy story (Kasper et al. 2002).

To this last point, it was neuroimaging and, in particular, PET (positron emission tomography) that would advance our understanding of antipsychotic dosing. Initiated in the 1980’s and in place at different sites by the 1990’s, PET work was able to establish thresholds for $D_2$ occupancy in terms of both therapeutic efficacy and side effects (EPS, extrapyramidal symptoms). Specifically, it was demonstrated that blockade of approximately 60-70% of $D_2$ receptors is required to optimize clinical efficacy, while levels exceeding 80% were associated with increased risk of EPS (Kapur et al. 2001). Moreover, levels exceeding 80% were not associated with clinical benefits that might warrant the increased risk of side effects.
Antipsychotics and Pharmacotherapy

APDs represent the mainstay of psychiatric treatment in psychotic disorders such as schizophrenia, and can be divided into two classes: dopamine receptor antagonists (first-generation neuroleptics) and dopamine-serotonin antagonists (second-generation neuroleptics). Dopamine receptor antagonists, alternatively referred to as classical or typical antipsychotics, were first introduced in the 1950s with chlorpromazine (Healy 2002). These drugs treat psychosis by blocking transmission in dopamine dense areas of the brain such as the striatum. While typical APDs are able to treat the positive symptoms of schizophrenia, they are not particularly effective against negative/cognitive symptoms. Moreover, high D₂ antagonism can cause various movement disorders including tardive dyskinesia (TD), akathisia, and dystonia.

In the late 1980’s, clozapine was identified as being uniquely efficacious in refractory or treatment resistant schizophrenia (Kane et al. 1988), and efforts turned to establish the underlying mechanisms that might account for this ‘atypicality’. Work by Meltzer and colleagues underscored the potential importance of clozapine’s concomitant serotonin 5-HT₂ antagonism (Meltzer et al. 1989), giving rise to a new generation of antipsychotics classified as novel, atypical, or second generation and characterized as dopamine-serotonin antagonists.
Figure 2 permits a comparison in terms of receptor binding profiles of the prototypical high-potency conventional D$_2$ antagonist (haloperidol) and a dopamine-serotonin antagonist such as clozapine. Atypical antipsychotics’ lower affinity for D$_2$ receptors, combined with their serotonergic modulation of dopamine (for example, at the level of the nigrostriatal pathway) translates to a lower risk of motor side effects (Karl et al. 2006) without jeopardizing treatment efficacy.

**Figure 2.** Pie charts of receptor affinity of antipsychotic drugs (www.medscape.com).
Antipsychotic Dosing: How Much and How Often?

The issue of antipsychotic dosing has already been touched upon in the context of how much drug should be administered; as has been pointed out, considerable gains were achieved in this regard through the use of PET to establish thresholds related to therapeutic efficacy and side effects, specifically EPS, based on D₂ occupancy thresholds.

There is, though, another component to antipsychotic dosing related to how often the drug needs to be administered. Both are important, especially given the burdensome side effects associated with antipsychotics; ideally, the goal of antipsychotic dosing is that of achieving the optimal therapeutic clinical dose while balancing this against a dose which minimizes risk of side effects.

Historically, antipsychotic treatment has been predicated on peripheral pharmacokinetics, with the goal of achieving and maintaining steady state levels. The notion of alternative dosing strategies to chronic continuous administration has as a rule been dismissed, at least in part related to the real problem of medication nonadherence in this population. More recently, the field demonstrated a willingness to revisit how much antipsychotic medication is required, although this really was only achieved in the face of compelling and clearly articulated PET data. At least to this point, frequency of APD administration has, for the most part, remained unexplored territory. Arguably, in
terms of a drug’s action and response, dose tells only part of the story (i.e. pharmacokinetics), but is not particularly informative in terms of pharmacodynamics (Boses & Manschrek 2002).

Only more recently has more attention turned to this particular issue, fueled by a number of findings and concerns. One such concern, obvious to clinicians who must administer these medications as well as those who must take them, is their troublesome side effect profile. While it might be argued that the newer antipsychotics have made gains in terms of EPS liability, these drugs are not without their own side effects. In particular, weight gain and metabolic sequelae have been linked to the newer antipsychotics (Newcomer et al. 2006), adding to the already increased mortality risk known to exist for individuals with serious mental illness (Laursen 2011).

Clinical observation also encourages a re-evaluation of old notions regarding the relationship between antipsychotic administration and response. For example, Viguera and colleagues (1997) and, more recently, Harrow et al. (2012) have shown that relapse and APD discontinuation do not occur simultaneously. While various explanations may be posited to account for this discrepancy, at least one alternative holds that continuous blockade of dopamine may not be necessary to prevent the reoccurrence of psychosis in individuals who have been stabilized.
Current thinking conceptualizes schizophrenia as a heterogeneous group of disorders, yet the approach to treatment is monolithic, one that argues for the same strategy in all patients. Boshes and Manschrek (2002) suggest that the use of multiple dosing schedules, as opposed to one (i.e. continuous dosing), may prove more clinically relevant in the treatment of heterogeneous group illnesses.

Finally, from a pharmacological standpoint, Baldessarini et al. (1999) hypothesize that a continuous dosing strategy may actually be detrimental across time, inducing neuroadaptations that may play a role in decreasing APD efficacy. If it is possible that continuous exposure to APDs results in tolerance to their antidopaminergic effects, more research is needed that focuses on the relationship between treatment schedule and APD efficacy.

Taken together, there have been considerable changes in antipsychotic dosing vis-à-vis how much is administered. Different lines of investigation, however, also call for a reassessment of how often these medications are administered. At least part of this argument arises from the notion that continuous antipsychotic exposure may invoke changes biologically that translate to diminished response across time, a point of concern given that current recommendations call for continuous antipsychotic administration over an individual’s lifetime once the diagnosis of schizophrenia has been made. With this information in hand, the discussion now turns to evidence which may be seen as legitimizing such a concern.
Drug Tolerance

In conditions such as schizophrenia, APDs are administered on a chronic and continuous basis over many years. From a pharmacological perspective, different drugs taken repeatedly induce changes in the magnitude of the drug’s response as a function of exposure (Stewart et al. 1993). More specifically, with repeated administration those mechanisms that are directly or indirectly involved in a drug’s effect undergo neuroplastic changes, and it is these changes that are in part responsible for the increase (sensitization) or decrease (tolerance) of a drug’s effect (Stewart et al. 1993). Tolerance, in turn, is associated with reduced drug effect(s).

Under such conditions, optimal drug response can no longer be maintained and increasingly larger doses are required to produce the initial therapeutic effect. Although tolerance may phenotypically differ across individuals, there are certain characteristics of tolerance that are common to all cases (Meyer et al. 2005 2005). Firstly, tolerance can be reversed (Meyer et al. 2005). This means that with drug cessation, it is possible that tolerance will gradually diminish. Second, the magnitude of tolerance that develops is partially dependent on the pattern of drug administration (i.e. dose, frequency of administration) (Meyer et al. 2005). Additionally, some effects of a drug may develop tolerance while others might not (Meyer et al. 2005); for example, developing tolerance to the sedative effects of a drug but not its effects on digestion.
This 'uneven' development of tolerance can become troublesome when a drug's desired effects diminish while its unwanted effects do not. Under such conditions, in order to maintain therapeutic efficacy the amount of drug given needs to be increased. This process is depicted in Figure 3. - by increasing the amount of drug administered, the potential for adverse side effects is also increased (Preskorn 1996).

Figure 3 Increasing the dose to enhance the therapeutic benefit and percentage of response increases the expression of the drug's adverse effects (Preskorn 1996).

Several types of tolerance exist, each with its own distinct mechanisms (Meyer et al. 2005). For the sake of simplicity, three principal forms of tolerance will be
discussed: behavioural tolerance, metabolic tolerance, and pharmacodynamic
tolerance. In addition, tachyphylaxis is briefly discussed.

**Behavioural (Contingent) Tolerance**

In an attempt to further understand the mechanisms of tolerance, research has
addressed the concept of behavioural plasticity; that is, tolerance as an
experience-dependent process that involves a component of learning and
adaptation (Meyer et al. 2005) This is in line with Siegel's theory of context-
dependent tolerance; which is premised on Pavlovian conditioning, and has been
shown in his earlier work that examined the contribution of drug-associated
environmental cues on heroin overdose (Siegel et al. 1982). To summarize, the
study consisted of a ‘tolerance phase’ and a ‘testing phase’. During the tolerance
phase, rats were given 30 daily injections of either heroin or placebo. Group 1
received heroin in Room A and placebo in Room B, Group 2, alternatively,
received heroin in Room B and placebo in Room A, and Group 3 received
placebo in both Room A and B. During the testing phase, all rats were given a
lethal dose of heroin (15 mg/kg). Group 1 was administered heroin in the same
room in which heroin had previously been received (Room A), Group 2 was given
heroin in a different room from which they had previously received heroin (Room
A), and controls (Group 3) were given heroin in either room (Room A + B). Fatal
overdose was commonly observed in Group 2 and Group 3, with mortality rates
of 64% and 96%, respectively, which contrasts with the low death rate (32%) in
animals given heroin in the same drug-paired environment (Group 1). Such results may be indicative of context-dependent tolerance, that is, drug tolerance that can become conditioned to specific contextual cues present at the time of drug administration.

Closely intertwined with behavioural tolerance is habituation. Habituation is the simplest form of learning, and involves learning to not respond to a repetitive stimulus (Meyer et al. 2005). For instance, the first administration of a drug may impact behaviour but after repeated exposure to the drug, behaviour will be impacted increasingly less. Unlike tolerance, which may in part be a result of changes at the receptor level, habituation is more likely to be contextual as it disappears when the subject is exposed to a novel setting.

**Metabolic (Dispositional) Tolerance**

Metabolic tolerance refers to a decrease in drug availability at target tissues as a result of repeated drug administration (Meyer et al. 2005). This process occurs when the metabolism of a drug is increased via liver microsomal induction, thereby decreasing drug effect. For example, upon consuming alcohol, the liver increases production of an enzyme called, alcohol dehydrogenase, that metabolizes alcohol and subsequently, other potentially harmful compounds (e.g. ethanol). Following repeated alcohol consumption, the body compensates by producing increasingly more alcohol dehydrogenase, thereby metabolizing
alcohol at an increasing rate, which in turn progressively inhibits alcohol’s effects (Meyer et al. 2005).

**Pharmacodynamic Tolerance**

Pharmacodynamic tolerance occurs at the cellular level, in which the body compensates for continuous drug exposure by modifying the amount of receptors available for the drug to act on. Alcohol can be used as an example, in which chronic alcohol exposure can lead to a reduction in either the number of GABA receptors or their sensitivity. Alternatively, with continuous receptor antagonism, upregulation can occur; that is, an increase in the number of receptors (Meyer et al. 2005) which also may lead to a reduction in drug effect (Samaha et al. 2007; 2008) (e.g. increased dopamine D$_2$ receptors following chronic APD exposure). This process shares similarities with sensitization, which can be defined as an increase in pharmacologic response following repeated administration of a stimulus; most likely also resulting from an upregulation of receptors.

**Tachyphylaxis versus Tolerance**

Though tachyphylaxis and tolerance are often used interchangeably, it should be noted that their mechanisms differ. While both tachyphylaxis and tolerance lead to a reduction in drug efficacy, the rate at which response decreases and is restored varies between the two. Tachyphylaxis involves a rapid reduction in drug efficacy following repeated drug exposure over a short period of time
(Binder 2012). Efficacy can be restored if the drug administration is briefly held, but is unchanged by an increase in dosage. On the other hand, tolerance generally results in a slow loss of drug efficacy. Drug effect can be improved if the dose is increased or is given over short time intervals, but is not restored if treatment is temporarily discontinued (Binder 2012), which was shown by Nielsen et al. (1974) in their finding that tolerance could last 6 weeks after antipsychotic withdrawal.

**Antipsychotic Tolerance**

Although variable, the course of schizophrenia is generally characterized by symptoms that wax and wane across time, with periodic exacerbations to a level that constitutes a relapse (Lieberman et al. 2001). Although antipsychotics do not guarantee against relapse, there is compelling evidence that relapse rates increase dramatically in the face of antipsychotic discontinuation (Gitlin et al. 2001). Accordingly, current treatment recommendations for illnesses such as schizophrenia call for continuous antipsychotic exposure (i.e. maintenance treatment) even in the absence of symptoms to reduce risk of relapse (APA 2004).

As previously discussed, when drugs are administered continuously there is evidence that magnitude of response can be altered. In terms of antipsychotic treatment, it has been reported that dose may need to be increased over time
(Margolese et al. 2002), while other evidence has indicated that relapse can occur despite antipsychotic adherence (Agid et al. 2011). While other explanations may exist, both scenarios raise the possibility of tolerance as an alternative, possibly evolving in the context of continuous APD administration.

To better understand the behavioural and biological underpinnings, focus will now turn to existing preclinical evidence.

**Preclinical Evidence of Antipsychotic Tolerance**

**Behavioural Studies:**

**Conditioned Avoidance Response (CAR)**

CAR represents a behavioural test routinely used in the preclinical setting to establish putative antipsychotic potential in molecules of interest. Briefly, the CAR task involves an aversive stimulus (such as an electric shock) being paired with a neutral stimulus (i.e. tone). Upon initial presentation of the aversive stimulus (the unconditioned stimulus, US), rats will actively escape the electric shock by moving to an alternative maze arm (the unconditioned response, UR). When pairing a neutral stimulus, such as a tone (the conditioned stimulus, CS) with the US, rats will eventually learn to associate the CS as a predictor of the US. Therefore, after proper conditioning the tone (CS) elicits an avoidance response similar to when an electric shock (US) is presented (*Figure 4*). CAR is
known for its strong predictability of APD efficacy; the likelihood increases if it inhibits the association between the CS and US, without disrupting unconditioned escape responses (i.e. inability to escape US due to sedation or locomotor immobility).

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<td>US – Electric shock</td>
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<td>UR – Escape</td>
<td>CS + US → UR</td>
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<td>CR- Escape</td>
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**Figure 4.** The basic model of conditioned avoidance response (CAR).

Using this behavioural measure, Samaha et al. (2007; 2008) explored the effects of continuous haloperidol exposure in rats. It was found that haloperidol initially suppressed CAR relative to controls, although this was diminished over time, thereby demonstrating that with continued haloperidol exposure the efficacy of haloperidol decreased (Figure 5). This is attributed to the effects of haloperidol, and not habituation, as the vehicle group’s avoidance rate remained consistent across time.

(see following page for Figure 5)
These findings are not specific to Samaha et al.’s (2007) study, but have also been shown by several other groups as well (Li et al. 2010; Mead & Li 2010; Varvel et al. 2002). To examine this issue further, Samaha et al. (2008) studied the effects of chronic continuous and transient haloperidol treatment in rats within the same paradigm. While rats continuously treated with haloperidol showed an increase in avoidance across time (i.e. decreased efficacy), transient haloperidol administration strengthened the disassociation between the CS-US (increased efficacy) (Figure 6).
Figure 6. The effects of chronic and continuous versus chronic and transient haloperidol exposure on conditioned avoidance response across time (Samaha et al. 2008).

These results demonstrate that the schedule of administration can cause the same drug to produce contrasting responses.
Vacuous Chewing Movements (VCMs)

Vacuous chewing movements (VCMs) can be observed in rodents exposed to continuous antipsychotic treatment, and as such have been used as an animal model that represents a proxy for tardive dyskinesia (TD) in humans. Turrone et al. (2002; 2003a; 2003b) examined the relationship between VCM expression and APD dosing schedule, reporting that VCMs were observed in rats receiving haloperidol either continuously via osmotic mini-pumps or intermittently by means of daily subcutaneous injections. However, findings showed significantly higher levels of VCMs in animals continuously treated with haloperidol compared to animals given daily injections (Figure 7).

![Graph showing VCMs over weeks of treatment for subcutaneous injections and minipump infusions.]

**Figure 7.** Vacuous chewing movements (VCMs) in animals... (continued on following page)
given haloperidol intermittently (daily subcutaneous injections, 0.1 mg/kg-1.0 mg/kg) and continuously (osmotic mini-pump implantation, 0.1 mg/kg-1.0 mg/kg/day) across the course of an 8-week study (Turrone et al. 2003a).

The increase in VCMs exhibited by rats continuously, but not intermittently, administered haloperidol once again indicates that pharmacological changes may occur as a result of continuous antipsychotic exposure. These findings have important clinical relevance, as they suggest that continuous maintenance APD therapy, which is common practice in the treatment of schizophrenia, may increase risk of TD while transient administration does not. Of course, the question remains as to whether transient antipsychotic administration is as effective in maintenance treatment.

Aggression

Neuroleptics are said to decrease aggressive behaviour in a range of species, including humans and rodents (Navarro et al. 1993). As such, Navarro and colleagues examined the temporal course of tolerance development to haloperidol as a means of separating different aspects of APD action on social behaviour (Navarro et al. 1993). Aggression and locomotor activity were examined in isolated male mice confronted with anosmic and grouped conspecifics. Animals were evaluated 30 minutes or 24 hours following haloperidol administration (after a single injection 0.4 mg/kg IP, followed by a
series of 15 or 30 daily injections). When animals were tested 30 minutes following haloperidol administration, tolerance to haloperidol’s antiaggressive effects was not found (i.e. animals did not exhibit aggressive behaviour when confronted with conspecifics), although locomotor testing at this time-point showed a clear development of tolerance (increased mobility) with repeated haloperidol administration (both with 15 and 30 daily injections). Interestingly, when tested 24 hours after injection, tolerance to the antiaggressive effects of haloperidol became evident (increased aggressive behaviour), whereas hypolocomotion remained unchanged. Such a clear divergence in the temporal course of tolerance development to haloperidol’s effects (antiaggressive, hypolocomotor) confirms notions that differential tolerance can occur between various effects of the same drug, while also suggesting that these effects can be mediated, at least in part, by separate neurophysiological mechanisms (Navarro et al. 1993).

**Pharmacological Studies:**

The inhibition of amphetamine-induced locomotion by dopamine antagonism is another means of testing drugs with antipsychotic potential. Samaha and colleagues (2007; 2008) performed amphetamine testing on animals treated with haloperidol/olanzapine during a 12-day study. The effects of haloperidol weakened, as shown by the significant increase in amphetamine-induced
hyperlocomotor activity. In fact, the level of activity exhibited by the haloperidol treated rats eventually resembled the vehicle group (Figure 8).

Figure 8. Locomotor activity in rats administered vehicle, 0.25 mg/kg/day of HAL, 0.75 mg/kg/day of haloperidol, or 10 mg/kg/day of olanzapine on Day 2 and Day 12 of treatment during amphetamine testing, and Day 5 of haloperidol/olanzapine withdrawal (Samaha et al. 2007).
Other studies have found similar results with animals that had been chronically exposed to haloperidol responding as though they were administered an acute dose of amphetamine (Barrett et al. 1992). Interestingly, withdrawal from continuous, but not transient, raclopride administration in rats resulted in the inability of raclopride to suppress amphetamine-induced hyperlocomotion when raclopride is reintroduced (Barrett et al. 1992).

**Neurochemical Studies:**

**C-fos Expression:**

Differences between animals continually and intermittently treated with APDs have been found in terms of c-fos levels (Samaha et al. 2007; Samaha et al. 2008; Ushijima et al. 1995). It has been shown that rats transiently treated with haloperidol exhibit an increase in striatal c-fos messenger RNA (mRNA) expression in the caudate-putamen, while rats continuously exposed to haloperidol do not (Samaha et al. 2007; Samaha et al. 2008) (Figure 9).

(see following page for Figure 9)
These postsynaptic differences suggest that transient, but not continuous, antipsychotic exposure may initiate intracellular events such as gene regulation that may in part contribute to the maintenance of APD efficacy over time. One possible explanation for this is that constant disruption of dopamine function, as seen with continuous APD exposure, may initiate compensatory responses (e.g. D₂ receptor upregulation) that lead to dopamine supersensitivity, and in turn a decrease in the antidopaminergic effects of APDs (Samaha et al. 2008), which may in fact be prevented if these drugs were administered intermittently.

Kindling, a process in which repeated brain stimulation results in sensitization, has been associated with neuronal adaptation as measured by levels of mRNA for the c-fos gene (Stevens et al. 1997). Of note, such studies indicate that intermittent stimulation is more effective than continuous stimulation in the modification of neuronal activity (Post 1980). This enhancement of neuronal
adaptation found with transient treatment appears to be paralleled by the increased expression of c-fos in intermittently, but not continuously, treated animals (Samaha et al. 2007; Samaha et al. 2008).

Levels of Dopamine Metabolites:

In vivo microdialysis in freely moving animals has been used to examine changes in the levels of extracellular dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and norepinephrine (NE) in the nucleus accumbens during ongoing haloperidol treatment (Samaha et al. 2007; Samaha et al. 2008; Ushijima et al. 1995). Reduced HVA levels demonstrate biochemical tolerance, which has previously been shown in animals continuously exposed to haloperidol (Carey and DeVeauh-Geiss 1984; Kurachi et al. 1995; Samaha et al. 2007) (Figure 10).

(see following page for Figure 10)
Figure 10. Levels of dopamine, DOPAC, and HVA following 2 and 12 days of amphetamine testing in rats (Samaha et al. 2007).
Morphological Brain Changes Following Chronic APD Exposure:

There is evidence to suggest that chronic APD exposure may directly affect brain structure (Vernon et al. 2011; Wotanis et al. 2003). Preclinical work has found reductions in total brain volume, namely in the frontal cerebral cortex (Vernon et al. 2011) and thalamic hippocampal regions (Wotanis et al. 2003) in animals that received chronic APD administration. Such findings can perhaps be translated to clinical models, as examination of postmortem brains of persons with schizophrenia have also found significant structural abnormalities (e.g. lateral and third ventricle enlargement; Lawrie et al. 1998) and signs of cortical shrinkage (Crow et al. 1989; Harrison 1999; Heckers 1997; Narr et al. 2005; Pakkenberg 1987; Selemon 2001), which have further been confirmed by neuroimaging studies in schizophrenia patients (Cahn et al. 2002; Ho et al. 2011; Olabi et al. 2011). In line with these findings, brain volume changes have also been demonstrated in first-episode schizophrenia patients following withdrawal from atypical antipsychotic treatment (Boonstra et al. 2011). In fact, evidence was suggestive of volume decreases in the nucleus accumbens and caudate putamen during APD discontinuation, whereas volume increases were observed in patients who continued their APD treatment (Boonstra et al. 2011). Put simply, this body of evidence suggests that chronic APD treatment can lead to morphological changes in the brain; changes that, at this point, have not been explored for possible adverse consequences. Indeed, APDs may in fact contribute to the genesis of brain abnormalities seen in schizophrenia that are
often attributed to the illness itself (Moncrieff et al. 2010; Navari et al. 2009). Although caution should be exerted when translating these results to the clinical population, they at the very least urge us to explore the possibility that maintenance APD therapy may have effects that extend beyond neurochemistry and pharmacokinetics; effects that could potentially represent a key factor in furthering our understanding of the neuroanatomical changes seen in schizophrenia, which could in turn challenge the efficacy of continuous APD exposure.

Clinical Evidence of Antipsychotic Tolerance:

Supersensitivity Psychosis (SSP)

Chouinard et al. (1990; 1994) proposed that dopamine receptor supersensitivity is a result of chronic continuous APD administration, which in turn may lead to the development of supersensitivity psychosis (SSP). SSP is a drug-induced phenomenon that leads to an increased risk of psychotic exacerbation/relapse following APD withdrawal (Chouinard et al. 1990; 1994). ‘Discontinuation syndromes’ such as SSP and their potential to trigger psychotic relapse have been demonstrated in several works (Baldessarini et al. 1999; Goudie et al. 1999 & 2000), and may suggest that chronic APD administration can cause a shift from dopamine receptor antagonism to dopamine receptor supersensitivity (Rupniak et al. 1983). Indeed, it has been shown in animals treated chronically
with APDs, which inhibit movement, that psychomotor response to dopamine agonists can significantly increase (reduced APD effect) (Bedard et al. 2011; Samaha et al. 2007). These findings have been confirmed by preclinical dopamine binding studies which have shown that chronic $D_2$ receptor inhibition results in a dramatic increase in $D_2$ receptors (Figure 11), with upregulation of $D_2$ binding sites by as much as 67% in the striatum and nucleus accumbens (Hitri et al. 1978; Kashihara et al. 1986a; Kashihara et al. 1986b; Kashihara et al. 1986c; Kashihara et al. 1986d; Kashihara et al. 1986e; Samaha et al. 2007; Samaha et al. 2008; Tadokoro et al. 2011).

Figure 11. The effects of chronic and continuous versus chronic and transient haloperidol treatment on striatal dopamine $D_2$ receptor binding (Samaha et al. 2008).
Clinically, these results have strong implications as they may help us to better understand why there are schizophrenia patients that experience psychotic relapse despite our efforts to control their symptoms through chronic APD maintenance therapy; which, according to emerging evidence, may in fact increase the number of dopamine receptor binding sites and in turn worsen the positive ‘hyperdopaminergic’ symptoms of schizophrenia (Goto et al. 2012; Hitri et al. 1978).

**Extended Antipsychotic Dosing**

Remington et al. (2005; 2011) propose a variation of intermittent treatment called extended dosing. Unlike intermittent dosing, which reintroduces treatment at the first sign of psychotic relapse following dosing gaps of variable intervals as part of “drug holidays”, extended antipsychotic dosing entails fixed, finite gaps.

In a pilot study, Remington and colleagues (2005) decreased APD treatment in two phases, initially through the administration of APDs every second day instead of daily for the first 3 months, followed by extending treatment to every third day for the remaining 3 months. While there were no significant differences in response between the continuously treated and extended dosing patients, there was a subjective preference for the extended dosing schedule amongst the participants (Remington et al. 2005). It should also be noted that only 1 of the 13 subjects in the study relapsed, and this was a result of complete discontinuation
of treatment. Similar results have been reported by other groups that have found targeted intermittent therapy to be effective in at least 50% of patients (Gaebel et al. 2011; Kusumi et al. 2008), while also producing significantly fewer side effects and increasing treatment adherence when compared to continuous APD exposure (Gaebel et al. 2011).

Extended dosing decreases antipsychotic exposure by as much as 50% or more but acknowledges the critical role of regular D₂ blockade. What is less clear at present is the ‘window’ that can exist between dosing without treatment, a window which may theoretically vary both between and within individuals. It is more in keeping with the notion of personalized medicine and the notion of schizophrenia as a heterogeneous group of disorders that may be reflected in different dosing needs. Boshes et al. (2002) have posited that fixed, intermittent dosing offers a greater opportunity to improve antipsychotic response, reduce side effects, increase adherence, and decrease costs. While the preliminary data appear promising, this work is in its earliest stages.

**Rationale**

Different lines of investigation in the literature suggest that tolerance may be a consequence of continuous antipsychotic exposure, a clinically relevant issue as this is how these drugs are routinely administered in clinical practice. There is evidence of physiological and neurochemical adaptations in response to
repeated drug exposure that seems to translate to diminished responsivity (Post 1980), leading to the proposition that constant drug exposure can result in tolerance. Typically, such models focus on drugs of abuse, although there is a body of evidence supporting its development in the face of chronic antipsychotic exposure. Further to this point, it has been shown that animals continuously versus transiently exposed to APDs can exhibit a decreased response across time. Not surprisingly, this line of investigation is largely preclinical based on the measures required, but also the ethical challenges that are faced in undertaking this work in patients.

Following this approach, the present work is being undertaken to better understand the behavioural consequences of continuous versus intermittent antipsychotic exposure.

**Objectives**

**Objective 1:** To determine the effect of continuous (using osmotic-mini pump) versus intermittent (via daily subcutaneous injections) haloperidol exposure on locomotor activity in rats.

**Objective 2:** To determine the effect of continuous (using intramuscular decanoate injections) versus intermittent (via daily subcutaneous injections)
haloperidol exposure on catalepsy, locomotor activity, and exploratory behaviour in rats.

**Hypotheses**

**Hypothesis 1:** Animals given haloperidol continuously will demonstrate tolerance to the hypolocomotor effects of haloperidol, which will be expressed through an initial suppression of activity that will diminish with time. Animals receiving haloperidol intermittently by means of daily injections, will exhibit a decrease in locomotor activity that is sustained across time.

**Hypothesis 2:** Animals exposed to haloperidol continuously will demonstrate a tolerance-like effect by exhibiting a decrease in catalepsy with time, and an initial suppression of exploratory behaviour and locomotion that will diminish across the span of the study. Animals given haloperidol intermittently via daily injections will exhibit catalepsy as well as decreases in both exploratory behaviour and locomotor activity that are sustained.
Preliminary Work I:

Effect of Continuous Versus Intermittent Haloperidol Exposure on Locomotion.
Overview

APD tolerance, despite its potentially profound clinical implications, remains a relatively understudied area of research. Assuming that drug tolerance occurs after prolonged and repeated drug exposure, the present study examined, using a behavioural animal model, the effects of chronic-continuous and chronic-intermittent haloperidol (an APD) exposure on treatment response. As noted, research on APD tolerance is still in its earliest stages; therefore, using a small group of animals, our first step was to lay the groundwork for a suitable paradigm to model this effect. Two routes of haloperidol administration were included: 0.25 mg/kg/day via osmotic-mini pump and 0.08 mg/kg via daily subcutaneous injections. 0.25 mg/kg/day by means of osmotic mini-pump is premised on modeling clinically relevant in vivo dopamine D_2 occupancy levels (between 65% and 80% striatal D_2 receptors). Further, treatment via osmotic mini-pump in rats leads to continuous blockade of D_2 receptors, which mimics what is seen in the clinical population during antipsychotic maintenance therapy. 0.08 mg/kg via daily subcutaneous injections was chosen to reflect a variation of intermittent dosing known as extended dosing, a dosing schedule proposed by Remington et al. (2005, 2011) that implements fixed, finite gaps between administration while ensuring the maintenance of clinically relevant occupancy levels.

Open-field locomotor activity was measured at the early and late stages of a 22-day experiment. It was hypothesized that animals given haloperidol continuously
would become tolerant to the hypolocomotor effects of haloperidol, which would be exhibited by an increase in locomotion across time (Week 1 versus Week 3). Conversely, it was also hypothesized that animals receiving haloperidol intermittently by means of daily injections would show decreased locomotor activity between testing sessions, and in turn suggest an increase in the maintenance of APD response.

**Materials and Methods**

**Animals**

Adult male Sprague-Dawley rats (N = 9) (Charles River, Montreal, Quebec), weighing 200 – 225 g at the start of the experiment, were singly housed in (19x10.5x8 inches) transparent polycarbonate cages (Lab Products Inc., Seaforth, Delaware, USA) and maintained on a 12-hour/12-hour light/dark cycle in a temperature controlled room at 21±2°C with unrestricted access to food and water. Animals were housed in the animal facility at the Centre for Addiction and Mental Health for 1 week before use in experiments. All procedures were conducted with the approval of the Animal Ethics Committee at the Centre for Addiction and Mental Health and were in accordance with the Canadian Council on Animal Care.
Drugs

Haloperidol (Toronto Research Chemicals, Toronto, Ontario) (0.25 mg/kg/day via osmotic mini-pump or 0.08 mg/kg via daily subcutaneous injection) was dissolved in 2.5% glacial acetic acid/saline solution to a concentration of 1.6 mg/ml for treatment via osmotic mini-pump (Alzet model 2ML4 28-day drug delivery, Durect Corp., Cupertino, California) and for treatment via subcutaneous injection, a stock solution of 0.8 mg/ml was made daily with a 1% glacial acetic acid/saline solution which was further diluted with saline to a concentration of 0.08 mg/ml. The concentration of haloperidol required to deliver 0.25 mg/kg/day via osmotic mini-pump was calculated using the predicted rat weight at the mid-point of the treatment period and the known delivery rate of the osmotic mini-pump (2.5 µl/hour). Osmotic mini-pumps were filled with (2 ml total volume) with either haloperidol or vehicle. Subcutaneous haloperidol injection volumes were based on the weight of each rat as measured daily. Vehicle was a 1% glacial acetic acid solution that was diluted to match the pH level of the subcutaneous injection solution.

Drug Treatment Schedules

Haloperidol was administered either continuously via osmotic mini-pump (0.25 mg/kg/day for 22 days) or intermittently by means of daily subcutaneous injections (0.08 mg/kg). To match the groups in terms of handling, injection, and surgical procedures, haloperidol-continuous animals (n = 3) were given daily
vehicle subcutaneous injections, haloperidol-intermittent animals (n = 3) underwent osmotic mini-pump surgery with pumps containing vehicle solution, and control animals (n = 3) received daily vehicle subcutaneous injections and underwent vehicle mini-pump surgery.

**Osmotic Mini-pump Implantation**

The animals were handled daily and given one week to habituate to the animal facility before surgery. Under 2.5 % isoflurane anesthesia, a small portion of each animal's back was shaved and then sterilized with isopropyl alcohol and betadine. A small incision (2 cm wide) was made within the sterilized area and the connective tissue in the subcutaneous space was carefully loosened using surgical scissors. The osmotic mini-pumps were sterilized with 70% isopropyl alcohol and were then subcutaneously inserted to lie between the scapulae with the flow moderator facing away from the incision. The incision was closed using surgical sutures (SOFSILK, Covidien, Dublin). Post-operative animals were placed in a recovery cage before being transferred to their home cage.

**Plasma Sampling**

To confirm haloperidol levels remained consistent across the course of the study, a blood sample was collected from each rat for analysis of plasma haloperidol concentrations on Day 3, Day 7 and Day 21 of the experiment. Plasma sampling took place at the same time for each animal on all sampling days. Under 2.5%
isoflurane anesthesia, the tail of each animal was dipped in warm water (40 °C) for 1 minute. A butterfly needle was inserted into the lateral tail vein and blood (0.3ml) was collected into an eppendorf tube. After blood collection was complete, pressure was applied to stop further bleeding. Using a centrifuge, samples were spun down and plasma was removed from the eppendorf tube with an insulin needle. The serum was then frozen over dry ice. Plasma samples were sent to the laboratory at InterVivo Solutions Inc. (Mississauga, Ontario).

**Locomotor Activity**

Locomotor activity was measured on Day 1 and Day 22 of the experiment for one hour beginning at 1100hrs. Animals were given a subcutaneous injection of either haloperidol or vehicle 45 minutes prior to the locomotor activity session. The locomotor activity boxes were clear polycarbonate cages (27x48x20cm) equipped with a row of six photocell beams placed 3 cm above the floor of the arena. A computer was used to detect and record the number of photobeam interruptions (total beam breaks, total beam lengths, and top-plane beam breaks) which are designed to measure all activity, cage crossings, and rearing, respectively. The equipment was housed in a separate room from the colony room.
Statistical Analysis

Statistical analyses were performed using SPSS Version 20.0. For statistical comparisons, means were considered significantly different when \( p < 0.05 \). A one-way analysis of variance (ANOVA) was used to determine the effect of treatment on locomotor activity (total beam breaks, total beam lengths, top-plane beam breaks). A paired-sample t-test was run for each of the behavioural indices of locomotor activity (total beam breaks, total beam lengths, and top-plane beam breaks) to determine if there was a treatment effect. Evaluation of differences in haloperidol plasma concentration (Week 1, Week 2, Week 3) was performed by a 3x3 factorial repeated measures ANOVA, with group (continuous, intermittent, control) as the between-subject variable and sampling (Day 1, Day 2, Day 3) as the within-subject factor.

Results

Locomotor Activity

A one-way ANOVA indicated a significant effect of treatment (\( F = 9.042, p = 0.024 \)).
Total Beam Breaks (All Activity)

A paired-samples t-test was conducted to determine the effect of treatment (continuous versus intermittent) on total beam breaks (Week 1 versus Week 3). In haloperidol-continuous and control animals, there was a significant increase in total beam breaks across session (p<0.05), whereas there was a trend toward a significant decrease in total beam breaks in animals intermittently administered haloperidol (p>0.05) (Figure 12).

**Figure 12.** Total beam breaks in animals (N = 9) administered haloperidol either continuously (0.25 mg/kg via osmotic mini-pump for 21 days)(n = 3) or intermittently (0.08 mg/kg via daily subcutaneous injections)(n = 3), and controls (vehicle)(n = 3) across testing session (Week 1, Week 3). In haloperidol-continuous and control animals, there was a significant increase in total beam breaks across session (p<0.05); *p<0.05. Error bars represent the standard error of the mean for the group.
A paired-sample t-test was conducted to determine the effect of treatment (continuous versus intermittent) on total beam lengths. Animals receiving continuous haloperidol exposure exhibited a significant increase in total beam lengths \( p < 0.05 \), whereas there was a trend toward a significant decrease in total beam lengths in animals intermittently administered haloperidol \( p > 0.05 \) (Figure 13). Total beam lengths did not significantly differ across the course of the study for controls, \( p > 0.05 \).

**Figure 13.** Total beam lengths in animals \( (N = 9) \) administered haloperidol either continuously \( (0.25 \text{ mg/kg via osmotic mini-pump for 21 days})(n = 3) \) or intermittently \( (0.08 \text{ mg/kg via daily subcutaneous injections})(n = 3) \), and controls \( (\text{vehicle})(n = 3) \) across testing session \( (\text{Week 1, Week 3})… \)
In haloperidol-continuous animals, there was a significant increase in total beam lengths (p<0.01); **p<0.01. Error bars represent the standard error of the mean for the group.

**Top-plane Beam Breaks (Rearing)**

A paired-sample t-test was conducted to determine the effect of treatment (continuous versus intermittent) on top-plane beam breaks. Animals receiving continuous haloperidol exposure exhibited a significant increase in top-plane beam breaks p<0.05, whereas there was a trend toward a significant decrease in top-plane beam breaks in animals intermittently administered haloperidol p>0.05 (Figure 14). Top-plane beam breaks did not significantly differ across the course of the study for controls, p>0.05.

![Graph](image)

**Figure 14.** Total top-plane beam breaks in animals (N = 9) administered haloperidol either continuously (0.25 mg/kg …

(continued on following page)
via osmotic mini-pump for 21 days) (n = 3) or intermittently (0.08 mg/kg via daily subcutaneous injections) (n = 3), and controls (vehicle) (n = 3) across testing session (Week 1, Week 3). In haloperidol-continuous animals, there was a significant increase in total top-plane beam breaks (p<0.01); **p<0.01. Error bars represent the standard error of the mean for the group.

Plasma Levels

For rats treated both continuously (0.25 mg/kg/day via osmotic mini-pump) (n = 3) and intermittently (0.08 mg/kg subcutaneously) (n = 3) with haloperidol, plasma drug concentrations (Table 2) did not significantly differ across time (F = 1.74, p = 0.221).

![Mean Haloperidol Plasma Concentrations (ng/mL)]

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>BLQ</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>Continuous</td>
<td>2.21 ± 0.23</td>
<td>2.09 ± 0.44</td>
<td>2.00 ± 0.65</td>
</tr>
<tr>
<td>Intermittent</td>
<td>3.97 ± 1.47</td>
<td>3.15 ± 0.24</td>
<td>3.02 ± 0.20</td>
</tr>
</tbody>
</table>

* BLQ denotes below the lower level of quantitation (0.5 ng/mL)

Table 2. Concentrations of haloperidol determined in rat plasma samples.

Therefore, it was confirmed that the increase in activity seen in the haloperidol-continuous animals was not a result of attenuated drug concentration levels.
Furthermore, a 3x3 factorial repeated measures ANOVA (between-subject factor: treatment; with-subject factor: time) indicated a significant main effect of treatment ($F = 57.31, p < 0.0001$), with intermittently treated animals demonstrating significantly higher mean haloperidol plasma concentrations compared to continuously treated animals ($p < 0.05$) and controls ($p < 0.001$).
Preliminary Work II:

Establishing a Preclinical Paradigm to Model the Effect of Continuous Versus Intermittent Haloperidol Exposure on Catalepsy, Locomotion, and Exploratory Behaviour.
Overview

Based on the findings in Preliminary Work I, which demonstrated tolerance in animals receiving continuous haloperidol exposure, we decided to further investigate this issue. Given the small sample size of our initial study, we set out to replicate its findings with a larger sample size and additional outcome measures. As existing literature on preclinical APD tolerance is quite limited, we first aimed to determine a suitable dose and treatment schedule to model this effect. To establish this paradigm, a behavioural animal model was again used, but with the addition of catalepsy, exploratory behaviour and locomotor activity as outcome measures; the reasoning behind the selection of these measures will be discussed in greater detail below. In terms of the amount of drug administered, the effects of varying doses of haloperidol (0.08 mg/kg, 0.10 mg/kg, 0.15 mg/kg, 0.20 mg/kg, 0.25 mg/kg, 0.5 mg/kg subcutaneously, and 1.0 mg/kg/day intramuscularly) were tested at numerous time-points across all measures. When selecting dose it was taken into consideration that 1) in Preliminary Work I, a significant drug effect on locomotor activity using 0.08 mg/kg haloperidol subcutaneously was demonstrated, 2) haloperidol-induced catalepsy in rodents typically involves a minimum dose of 0.5 mg/kg subcutaneously (Ezrin-Waters et al. 1977) in order to meet the necessary threshold of dopamine D₂ receptor blockade (>80% occupancy) to induce catalepsy (Crocker et al. 2001; Wadenberg et al. 2000), 3) our group has previously examined an animal model of APD-induced EPS using 1.0 mg/kg/day haloperidol decanoate (Turrone et al. 2000).
and 4) 3 outcome measures needed to be observed in each animal, which would require a balance between administering enough drug to induce catalepsy, but not so much as to completely inhibit locomotion and exploratory behaviour. Regarding the rationale for the selection of outcome measures, the present work wanted to examine the effects of continuous versus intermittent haloperidol exposure using behavioural measures with high predictive validity for APD efficacy. Catalepsy, for instance, is a widely used animal model of APD-induced EPS (Hoffman & Donovan 1995). Also of note is that APDs are the most commonly studied ‘cataleptogens’, with the degree of catalepsy typically positively and negatively correlated with antipsychotic potency (Campbell et al. 1980; Sanberg et al. 1988) and striatal dopamine activity (Toru et al. 1985), respectively.

Exploratory behaviour was an included outcome measure due to its potential relationship with dopamine. For instance, previous reports have established that dopamine blockade (e.g. dopamine denervation) decreases exploratory behaviour, while dopamine agonism (e.g. apomorphine) increases novel object seeking (Fink 1980; Alttoa et al. 2009). Further, elevated extracellular dopamine levels and D2 receptors in the striatum have been observed in highly explorative animals (Alttoa et al. 2009). These findings suggest that dopamine may play a vital role in exploratory behavior, and based on this assumption, it was chosen to investigate whether modifying the amount of APD-induced dopamine antagonism (continuous versus intermittent) would alter exploratory activity. Additionally,
locomotor activity was included as an outcome measure given the tolerance-like effect that was discovered in Preliminary Work I.

**Materials and Methods**

**Animals**

Adult male Sprague-Dawley rats (N = 30) (Charles River, Montreal Quebec) previously used in an unrelated experiment, weighing 500-600 g, were singly housed in (19“x10.5”x8”) transparent polycarbonate cages (Lab Products Inc., Seaforth, Delaware, USA) and maintained on a 12-hour/12-hour light/dark cycle in a temperature controlled room at 21±2ºC with unrestricted access to food and water. All procedures were conducted with the approval of the Animal Ethics Committee at the Centre for Addiction and Mental Health and were in accordance with the Canadian Council on Animal Care.

**Drugs**

Haloperidol decanoate (Sandoz Canada, Boucherville, Quebec) (1 mg/kg/day intramuscularly injected) was diluted in a vehicle solution (sesame oil containing 50 µl/ml propanol alcohol) at a concentration of 30 mg/ml. The volumes administered intramuscularly were based on the animals’ anticipated mid-point weights. These mid-point weight calculations were derived from our preliminary
research (assuming 7 g daily weight gain). Haloperidol (Toronto Research Chemicals, Toronto, Ontario) 0.08 mg/kg, 0.10 mg/kg, 0.15 mg/kg, 0.20 mg/kg, 0.25 mg/kg, and 0.5 mg/kg via daily subcutaneous injections was dissolved in 1% glacial acetic acid/saline solution.

**Intramuscular Injections**

Under 2.5% isoflurane anesthesia, animals received an intramuscular injection of haloperidol decanoate into the quadriceps muscle group of the thigh. Animals were placed in a recovery cage before being transferred to their home cage.

**Drug Treatment Schedule**

Haloperidol was administered either continuously via haloperidol decanoate (1 mg/kg/day) or intermittently via subcutaneous injection (0.08 mg/kg, 0.10 mg/kg, 0.15 mg/kg, 0.20 mg/kg, 0.25 mg/kg, 0.50 mg/kg). Controls received either a vehicle intramuscular or subcutaneous injection.

**Exploratory Behaviour**

After a 5-minute habituation period in the arena (a custom-built black opaque cage, 20”x10”x12”) animals were removed and given a subcutaneous injection of either haloperidol (0.08 mg/kg – 0.50 mg/kg) or vehicle. Animals given haloperidol intramuscularly were also tested. Exploratory behaviour was studied
at numerous time-points (5, 10, 20, 30, 45, and 60 minutes post-injection). A novel object (building blocks and shapes) was positioned at the far end of each arena and was secured with Velcro®. The objects used were easy to clean and were wiped with 70% ethanol to remove odors between sessions. To prevent coercion to explore the novel object, animals were placed on the opposite end of the arena facing away from the novel object. Their activity was recorded by EthoVision software (Noldus, The Netherlands) and a Sony CCD video camera. Digitally, a rectangular zone was created around each object as a means of tracking the duration, and latency to explore the object. Object exploration was defined as entrance into the novel object zone. Using backup video footage, the experimenter carefully watched each trial for tracking errors. Objects were removed at the end of each session.

**Locomotor Activity**

Following the exploratory session, locomotor activity was measured. Both the timing between sessions (exploratory and locomotor) and duration of locomotor testing were tested across numerous time intervals. Locomotor activity was measured for 15, 20, 25, or 30 minutes in a (20”x10”x12”) custom-built black opaque arena. Locomotor activity was recorded by EthoVision software (Noldus, The Netherlands) and a Sony CCD video camera. The equipment was housed in a separate room from the colony room.
**Catalepsy**

Catalepsy was assessed 45 minutes, 60 minutes, and 120 minutes post-injection (haloperidol or saline) in the same room in which exploratory behaviour and locomotor activity were measured. Separately, each rat was gently placed over the edge of a platform raised 9 cm above the working surface and slowly released by the experimenter. Catalepsy was scored as the latency of the rat to remove both of its forepaws from this fixed position or to climb onto the platform. Each trial was a maximum of 180 seconds in duration.

**Results**

**Dose**

A range of doses across varying treatment schedules were tested to determine if it was possible to observe each of the selected outcome measures (catalepsy, locomotor activity, and exploratory behaviour) within a single paradigm. While there was no difficulty in inducing these behaviours separately, it was particularly difficult to obtain each of these measures in a single testing session. When administering haloperidol subcutaneously, doses greater than 0.10 mg/kg (i.e. 0.15 mg/kg, 0.20 mg/kg, 0.25 mg/kg, and 0.5 mg/kg) completely inhibited locomotion and exploratory behaviour, whereas doses less than 0.10 mg/kg (i.e. 0.08 mg/kg), did not induce catalepsy at any time point (i.e. 45 minutes, 60 minutes, and 120 minutes post-injection). Animals that received haloperidol...
decanoate intramuscularly (1.0 mg/kg/day) demonstrated measurable levels of exploratory behaviour, hypolocomotion and catalepsy at all time-points.

**Timing**

Once the dose for the haloperidol-intermittent group (0.10 mg/kg) was selected, the timing of the treatment schedule was fine tuned, which was found to have an enormous impact on locomotor and exploratory behaviour; where locomotor and exploratory activity were both completely suppressed approximately 15-20 minutes post-injection (0.10 mg/kg haloperidol subcutaneously), and catalepsy was expressed only after a minimum of 45 minutes post-injection, with its peak response at 120 minutes post-treatment. These observations formed the basis of our behavioural model, which is discussed in further detail in the proceeding methods section of Experiment 1.
Experiment 1:

Effect of Continuous versus Intermittent Haloperidol Exposure on Catalepsy, Exploratory Behaviour, and Locomotor Activity.
Overview

Using Preliminary Work I & II as the foundation for Experiment 1, the present work examined the effect of continuous and intermittent haloperidol exposure on catalepsy, locomotor activity, and exploratory behaviour. Based on our earlier observations (Preliminary Work II), two routes of haloperidol administration were chosen: 1.0 mg/kg/day intramuscularly for 21 days and 0.1 mg/kg via daily subcutaneous injections. To reiterate, haloperidol decanoate administered at 1.0 mg/kg/day was selected to model chronic-continuous APD exposure in humans, and as discussed in Preliminary Work II, this dose induced catalepsy while maintaining measurable levels of hypolocotion and exploratory behaviour. 0.10 mg/kg via daily subcutaneous injections was chosen to reflect extended dosing. In this work, the dose (0.1 mg/kg) was increased slightly from Preliminary Work I (0.08 mg/kg), as haloperidol-induced catalepsy was not found in doses less than 0.1 mg/kg (Preliminary Work II), which has been confirmed by earlier investigations (Kapur et al. 2003). Given that multiple measures were to be obtained from each animal, the lowest possible dose that could induce catalepsy while not completely inhibiting exploratory behaviour and locomotor activity was administered (Preliminary Work II).

It was hypothesized that animals given haloperidol continuously would show a decrease in catalepsy across time, that is, a decrease in muscular rigidity, and an increase in exploratory behaviour and locomotion. It was further hypothesized
that animals receiving haloperidol intermittently by means of daily subcutaneous injections would remain cataleptic throughout the study’s duration, with locomotor and exploratory behaviour also consistently being depleted.

**Materials and Methods**

**Animals**

Adult male Sprague-Dawley rats (N = 24) (Charles River, Montreal, Quebec), weighing 200 – 225 g at the start of the experiment, were singly housed in (19”x10.5”x8”) transparent polycarbonate cages (Lab Products Inc., Seaforth, Delaware, USA) and maintained on a 12-hour/12-hour light/dark cycle in a temperature controlled room at 21±2ºC with continuous access to food and water. Animals were handled daily and left for 1 week to acclimatize before the experiment commenced. All procedures conformed to the guidelines of the Canadian Council on Animal Care and were approved by the Centre for Addiction and Mental Health (CAMH) Animal Care Committee.

**Drugs**

Haloperidol decanoate (Sandoz Canada, Boucherville, Quebec) (1 mg/kg/day for 21 days via a single intramuscular injection) was diluted in a vehicle solution (sesame oil containing 50 µl/ml propanol alcohol) at a concentration of 30 mg/ml.
The volumes administered intramuscularly were based on the animals’ mid-point weights. These mid-point weight calculations were derived from our preliminary research (assuming 7 g daily weight gain). Calculations were adjusted as needed based on the animals’ weights when they arrived to the facility. The sesame oil/propanol alcohol solution used to dilute haloperidol decanoate was administered intramuscularly to control animals and injection volumes were matched to the treatment group. Haloperidol (Toronto Research Chemicals, Toronto, Ontario) 0.10 mg/kg via daily subcutaneous injection was dissolved in 1% glacial acetic acid/saline solution. Animals that received vehicle subcutaneous injections were injected with a 1% glacial acetic acid solution that was diluted to match the pH level of the haloperidol subcutaneous injection solution.

**Intramuscular Injections**

Under 2.5% isoflurane anesthesia, animals received an intramuscular injection of either haloperidol decanoate or sesame oil vehicle into the quadriceps muscle group of the thigh. Animals were placed in a recovery cage before being transferred to their home cage.

**Drug Treatment Schedule**

Haloperidol was administered either continuously via a single intramuscular
injection of haloperidol decanoate (1 mg/kg/day for 21 days) or intermittently by means of daily subcutaneous injections (0.10 mg/kg) for 21 days. To match the groups in terms of handling, injection, and surgical procedures, haloperidol-continuous animals (n = 8) were given daily vehicle subcutaneous injections, haloperidol-intermittent animals (n = 8) received an intramuscular injection containing sesame oil vehicle, and control animals (n = 8) received daily vehicle subcutaneous injections and received a vehicle intramuscular injection.

**Exploratory Behaviour**

Exploratory behaviour was measured on Day 3, Day 14, and Day 21 of the experiment for 5 minutes beginning at 0930hrs. After a 5-minute habituation period in the arena (20"x10"x12"), a custom-built black opaque cage) animals were removed and given a subcutaneous injection of either haloperidol (haloperidol-intermittent) or vehicle (haloperidol-continuous, controls). A novel object (building blocks and shapes) was positioned at the far end of each arena and was secured with Velcro®. The objects used were easy to clean and were wiped with 70% ethanol to remove odors between sessions. 5 minutes following injection, to prevent coercion to explore the novel object, animals were placed on the opposite end of the arena facing away from the novel object. Their activity was recorded by EthoVision software (Noldus, The Netherlands) and a Sony CCD video camera. Digitally, a rectangular zone was created around each object as a means of tracking the frequency, duration, and latency to explore the object.
Object exploration was defined as entrance into the novel object zone. Using backup video footage, the experimenter carefully watched each trial for tracking errors. Objects were removed at the end of each session.

**Locomotor Activity**

Immediately following exploratory behaviour testing, locomotor activity was measured for 30 minutes beginning at 1000hrs in a (20"x10"x12") custom-built black opaque arena. Locomotor activity was recorded by EthoVision software (Noldus, The Netherlands) and a Sony CCD video camera. The equipment was housed in a separate room from the colony room.

**Catalepsy**

Catalepsy was assessed 120 minutes post-injection (haloperidol or saline) in the same room in which exploratory behaviour and locomotor activity was measured. Separately, each rat was gently placed over the edge of a platform raised 9 cm above the working surface and slowly released by the experimenter. Catalepsy was scored as the latency of the rat to remove both of its forepaws from this fixed position or to climb onto the platform. Each trial was a maximum of 180 seconds in duration. Catalepsy was tested on Day 3, Day 7, Day 21 following the measurement of exploratory behaviour and locomotor activity.
Statistical Analysis

Statistical analyses were performed using SPSS Version 20.0. For statistical comparisons, means were considered significantly different when $p < 0.05$. The data from each outcome measure (catalepsy, locomotor activity, exploratory behaviour) were separately analyzed using a 3x3 factorial repeated measures analysis of variance (ANOVA), with treatment (continuous, intermittent, control) as the between-subject variable and time (Week 1, Week 2, Week 3) as the within-subject factor, followed by independent/repeated measures t-tests for post-hoc within- and between-group comparisons where appropriate. It should be noted that one animal from the control group was excluded from the statistical analyses, as the animal’s behaviour was untypical of a healthy control (e.g. the excluded animal exhibited minimal locomotor activity or exploratory behaviour, which was comparable to haloperidol-treated animals).
Results

Exploratory Behaviour

Total duration of time spent in novel object-paired zone

A 3x3 factorial repeated measures ANOVA (between-subject factor: treatment; within-subject factor: time) indicated a significant main effect of time (F = 3.610, p = 0.047), no main effect of treatment (F = 1.435, p = 0.262), and a significant time x treatment interaction (F = 3.152, p = 0.024). When paired t-tests were performed (comparing total duration of time spent in a novel object-paired zone on Week 1 vs. Week 2, Week 1 vs. Week 3, and Week 2 vs. Week 3), it revealed no significant differences in controls (p = 0.616, p = 0.779, p = 0.428, respectively) or haloperidol-intermittent animals (p = 0.301, p = 0.743, p = 0.285, respectively), and significant differences, that is, an increase in total duration of time spent in the novel object-paired zone, in haloperidol-continuous animals across each of the three comparisons (p = 0.043, p = 0.004, p = 0.001, respectively).

(see following page)
Figure 15. Total duration of time spent in a novel object-paired zone (seconds) in animals (N = 23) administered haloperidol either continuously (1.0 mg/kg/day intramuscularly for 21 days)(n = 8) or intermittently (0.10 mg/kg via daily subcutaneous injections)(n = 8), and controls (vehicle)(n = 7) across testing session (Week 1, Week 2, Week 3). A significant effect of time (p<0.05) and a significant time x treatment interaction (p<0.05) were found. Paired t-tests were performed for each group, comparing total duration of time spent in a novel object-paired zone on Week 1, Week 2, and Week 3; * p<0.05. Error bars represent the standard error of the mean for the group.

**Latency of entry to novel object-paired zone**

A 3x3 factorial repeated measures ANOVA (between-subject factor: treatment; within-subject factor: time) indicated a significant main effect of time (F = 8.694, p = 0.002), no main effect of treatment (F = 2.171, p =0.140), and no significant time x treatment interaction (F = 0.742, p = 0.489). When paired t-tests were performed (comparing latency of entry to a novel object-paired zone on Week 1 vs. Week 2, Week 1 vs. Week 3, and Week 2 vs. Week 3), it revealed no
significant differences in controls between Week 1 and Week 2 (p = 0.086), or Week 2 and Week 3 (p = 0.616), but demonstrated a significant decrease in latency between Week 1 and Week 3 (p = 0.047). This effect was also found in haloperidol-intermittent animals, where the latency of entry significantly decreased between Week 1 and Week 3 (p = 0.032). In haloperidol-continuous animals, there was a trend that approached the statistical threshold of significance, toward a significant decrease in latency between Week 1 and 3 (p = 0.083). All other comparisons were non-significant.

**Figure 16.** Latency of entry to a novel object-paired zone (seconds) in animals (N = 23) administered haloperidol either continuously (1.0 mg/kg/day intramuscularly for 21 days)(n = 8) or intermittently (0.10 mg/kg via daily subcutaneous injections)(n = 8), and controls (vehicle)(n = 7) across testing session (Week 1, Week 2, Week 3). There was a significant effect of time (p<0.01). Paired t-tests were performed for…

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each group, comparing latency of entry to a novel object-paired zone on Week 1, Week 2, and Week 3;* p<0.01. Error bars represent the standard error of the mean for the group.

**Locomotor Activity**

**Mean Velocity**

A 3x3 factorial repeated measures ANOVA (between-subject factor: treatment; within-subject factor: time) indicated no main effect of time (F = 1.1261, p = 0.294), a significant main effect of treatment (F = 15.323, p = 0.0001), and no significant time x treatment interaction (F = 2.479, p = 0.644). Paired t-tests were performed for each group, comparing mean velocity (cm/s) on Week 1, Week 2, and Week 3. When paired t-tests were performed (comparing mean velocity on Week 1 vs. Week 2, Week 1 vs. Week 3, and Week 2 vs. Week 3), it revealed no significant differences in controls (p = 0.198, p = 0.832, p = 0.111, respectively), haloperidol-continuous animals (p = 0.215, p = 0.148, p = 0.494, respectively) or haloperidol-intermittent animals (p = 0.732, p = 0.710, p = 0.305, respectively).

(see following page)
Figure 17. Mean velocity (cm/s) in animals (N = 23) administered haloperidol either continuously (1.0 mg/kg/day intramuscularly for 21 days)(n = 8) or intermittently (0.10 mg/kg via daily subcutaneous injections)(n = 8), and controls (vehicle)(n = 7) across testing session (Week 1, Week 2, Week 3). There was a significant effect of treatment (p<0.001). Paired t-tests were performed for each group, comparing mean velocity on Week 1, Week 2, and Week 3. Error bars represent the standard error of the mean for the group.

Total Distance Travelled

A 3x3 factorial repeated measures ANOVA (between-subject factor: treatment; within-subject factor: time) indicated a significant main effect of time (F = 20.608, p = 0.0001), a significant main effect of treatment (F = 4.084, p = 0.033), and no significant time x treatment interaction (F = 1.689, p = 0.173). Paired t-tests were performed for each group, comparing total distance travelled on Week 1, Week
2, and Week 3. When paired t-tests were performed (comparing total distance travelled on Week 1 vs. Week 2, Week 1 vs. Week 3, and Week 2 vs. Week 3), it revealed significant differences in controls, where a significant increase in the total distance travelled was found across each comparison (p = 0.007, p = 0.010, p = 0.026, respectively), a significant increase in the total distance travelled by haloperidol-intermittent animals when comparing Week 1 and 2 and Week 1 and 3, but not Week 2 and 3 (p = 0.010, p = 0.049, p = 0.855, respectively), and significant differences, that is, an increase in the total distance travelled, in haloperidol-continuous animals when comparing Week 1 and 2 (p = 0.010), but not Week 1 and 3 and Week 2 and 3 (p = 0.055, p = 0.212, respectively).

Figure 18. Total distance travelled (cm) in animals (N = 23) administered haloperidol either continuously (1.0 mg/kg/day intramuscularly for 21 days)(n = 8) or intermittently...

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(0.10 mg/kg via daily subcutaneous injections)(n = 8), and controls (vehicle)(n = 7) across testing session (Week 1, Week 2, Week 3). There was a significant effect of treatment (p<0.05) and time (p<0.01). Paired t-tests were performed for each group, comparing total distance travelled on Week 1, Week 2, and Week 3; * p<0.05, **p<0.01. Error bars represent the standard error of the mean for the group.

**Catalepsy**

A 3x3 factorial repeated measures ANOVA (between-subject factor: treatment; within-subject factor: time) did not find a main effect of time (F = 0.782, p = 0.387), but indicated a significant main effect of treatment (F = 13.276, p = 0.0001), and a significant time x treatment interaction (F = 21.223, p = 0.001). Paired t-tests were performed for each group, comparing mean catalepsy scores on Week 1, Week 2, and Week 3. When paired t-tests were performed (comparing mean catalepsy scores on Week 1 vs. Week 2, Week 1 vs. Week 3, and Week 2 vs. Week 3), it revealed a significant difference in controls, where a significant increase mean catalepsy scores was between Week 1 and Week 3 (p = 0.047) but not between Week 1 and 2, and Week 2 and 3 (p = 0.074, p = 0.907, respectively), a significant increase in mean catalepsy scores by haloperidol-intermittent animals when comparing Week 1 and 3 (p = 0.004), but not Week 1 and 2, or Week 2 and 3 (p = 0.060, p = 0.069, respectively), and significant differences, that is, a decrease in mean catalepsy scores, in
haloperidol-continuous animals when comparing Week 1 and 2 (p = 0.017) and Week 1 and 3 (p = 0.011), but not Week 2 and 3 (p = 0.356).

**Figure 19.** Mean catalepsy scores (seconds) in animals (N = 23) administered haloperidol either continuously (1.0 mg/kg/day intramuscularly for 21 days) (n = 8) or intermittently (0.10 mg/kg via daily subcutaneous injections) (n = 8), and controls (vehicle) (n = 7) across testing session (Week 1, Week 2, Week 3). In haloperidol-continuous animals, catalepsy significantly decreased from Week 1 to Week 3 (p<0.01), while a significant increase in catalepsy between Week 1 and Week 3 was found in haloperidol-intermittent animals and controls (p<0.01 and p<0.05, respectively). Paired t-tests were performed for each group, comparing mean catalepsy scores on Week 1, Week 2, and Week 3; * p<0.05, **p<0.01. Error bars represent the standard error of the mean for the group.
General Discussion

Summary:

In the present work, a behavioural animal model of APD tolerance was investigated. Our preliminary research (Preliminary Work I) examined locomotor differences in animals continuously and intermittently administered haloperidol, an APD known for its motor inhibiting properties. Over the course of 3 weeks, male Sprague-Dawley rats received haloperidol either intermittently by means of daily subcutaneous injections (0.08 mg/kg) or continuously via osmotic mini-pump (0.25 mg/kg/day); doses chosen reflected clinically relevant levels of dopamine D₂ receptor antagonism (Kapur et al. 2003). It was of particular interest to determine whether the hypolocomotor effects of haloperidol, a well-established indicator of APD response, would be expressed differently in animals chronically administered haloperidol continuously versus intermittently. As hypothesized, animals administered haloperidol continuously via osmotic mini-pump showed a significant increase in locomotor activity across testing sessions (Week 1 and Week 3), suggestive of tolerance. There was also a trend towards a significant decrease in locomotor activity in animals intermittently exposed to haloperidol. Controls showed a significant increase in locomotion, albeit to a lesser degree than the haloperidol-continuous group.
To follow-up on this preliminary work, it was investigated in greater detail the possibility of tolerance resulting from chronic and continuous APD exposure. First, additional preliminary studies (Preliminary Work II) were conducted to establish a suitable paradigm. The effect of continuous compared to intermittent haloperidol exposure was again examined using a behavioural animal model, but with additional outcomes measures: catalepsy, exploratory behaviour, and locomotor activity (Experiment 1). These measures were selected because of evidence linking an impact of antipsychotics on these behaviours, thought to at least in part to be related to $D_2$ activity. Two routes of administration were included: 1.0 mg/kg/day via an intramuscular injection of haloperidol decanoate and 0.1 mg/kg via daily subcutaneous injections. Haloperidol decanoate, a depot formulation, was selected to mirror continuous APD administration and was administered at 1.0 mg/kg/day based on earlier work done in this lab that successfully examined locomotor impairments in rats using this given formulation and dose (Turrone et al. 2003). Further, haloperidol decanoate administered at 1.0 mg/kg/day in rodents establishes $D_2$ occupancy of ~84% (Turrone et al. 2003), which approximates levels observed in humans and permits measurement of both locomotion and catalepsy (i.e. it allows for locomotor activity). A dose of 0.10 mg/kg via daily subcutaneous injections was chosen, as this dose has been demonstrated to establish $D_2$ occupancy levels of ~83% (Turrone et al. 2003). Once again, this dose permitted multiple behavioural measures (a higher dose induces a level of catalepsy that interferes with exploratory behaviour and locomotor activity).
Thus, in Experiment 1 our preliminary research was expanded on by examining the effect of continuous versus intermittent haloperidol exposure on multiple, well-established, behavioural indices of APD efficacy. It was found that 1) catalepsy significantly and progressively increased across time in animals intermittently treated with haloperidol, whereas catalepsy significantly decreased (again, suggestive of tolerance) with continuous haloperidol exposure, 2) exploratory behaviour, as measured by latency of entry to, and total time spent in, a novel object-paired zone, was inconsistent in that evidence of tolerance was shown in the latter and not the former. To summarize, total time spent in the novel object-paired arm dramatically increased across sessions in the haloperidol-continuous group but not in the haloperidol-intermittent group or controls. On the other hand, latency of entry to a novel object-paired zone decreased in all groups, although it can be argued that this change was more notable in the haloperidol-continuous group, 3) across time, treatment produced no significant effects on locomotor activity (total distance travelled, mean velocity) although once again there was a trend toward a significant increase in mean velocity in the haloperidol-continuous animals. Of note, all three groups showed an increase in the total distance travelled across testing session.

Summarizing, findings from the work offer at least partial support for behavioural tolerance in the face of continuous versus intermittent antipsychotic exposure. At the same time results were inconsistent, giving credence to the notion that tolerance may also be ‘uneven’ and behavior-specific. Put simply, the evidence
highlights that tolerance is very likely a complex, multi-factorial phenomenon, most likely involving multiple mechanisms operating at different levels of organization.

Comparison to Existing Literature:

Effect on Catalepsy

Catalepsy is a frequently used preclinical index of APD-induced EPS, with the degree of catalepsy exhibited said to be a function of dopamine D_2 receptor occupancy within the striatum and substantia nigra (Crocker et al. 2001). While haloperidol-induced catalepsy in rodents following acute haloperidol administration is firmly rooted in the literature, the effect of chronic haloperidol exposure on catalepsy is not as clear.

Tolerance to the cataleptic-inducing effects of APDs, that is a decrease in muscular rigidity, has been reported. This body of evidence has shown that animals treated daily with haloperidol (Asper et al. 1973; Campbell et al. 1981; Ezrin-Waters et al. 1977) compared to weekly (Barnes 1990), (Figure 23) demonstrate a dramatic decrease in catalepsy (Asper et al. 1973; Barnes et al. 1990; Campbell et al. 1981; Ezrin-Waters et al. 1977) and increase in apomorphine stereotypies (Asper et al. 1973). These findings have been attributed to a loss of sensitivity of the striatal neurons following chronic APD exposure (Asper et al. 1973); although, this may only be one part of the story. To
at this point, there is evidence that learning may also be involved in APD tolerance; for example, preclinical studies have found behavioural tolerance to the cataleptic effects of APDs in rats tested with haloperidol in a haloperidol-paired environment but not in a saline-paired environment (De Graaf et al. 1986; Hinson et al. 1982).

![Figure 20](image)

**Figure 20.** Catalepsy scores during daily or weekly administration of haloperidol 0.15 mg/kg or 1.5 mg/kg subcutaneously (Barnes et al. 1990).

That multiple factors may play a role is in line with the finding that tolerance to the cataleptic effects of APDs has been inconsistent, found in some instances (Barnes et al. 1990; Carey et al. 1984; De Graaf et al. 1986; Ezrin-Waters et al.
1977; Nsimba 2009) but not others (Antelman et al. 1986). Such discrepancies may, in part, be due to procedural and technical differences across studies (Carey et al. 1984). For example, schedule of APD administration (e.g. daily versus weekly injections) may play a role (e.g. tolerance versus sensitization) over a prolonged period (Carey et al. 1984; Barnes et al. 1990). Other factors may influence cataleptic response include frequency and method of testing catalepsy (Carey et al. 1984). For instance, following repeated catalepsy testing Barnes and colleagues found that catalepsy decreased when measured with the inclined screen test but was unaffected when using the horizontal bar test (Barnes et al. 1990). Taking this into consideration, future research may wish to measure catalepsy using multiple tests and drug schedules over an extended interval.

While evidence of tolerance, as measured by catalepsy, was observed in the haloperidol-continuous group here (Experiment 1), results are somewhat inconsistent with earlier reports as daily subcutaneous injections of haloperidol led to an increase in catalepsy, at odds with other reports that have found tolerance following repeated daily injections (Asper et al. 1973; Barnes et al. 1990; Campbell et al. 1984; Ezrin-Waters et al. 1977). Again though, different factors influencing the expression of catalepsy could account for the differences seen across studies (e.g. dose, drug administration schedule, route of administration, measure of catalepsy). For example, Barnes and colleagues examined catalepsy across several conditions (e.g. inclined screen test versus
horizontal bar test, daily versus weekly administration, single versus repeated testing, 0.05-5.00 mg/kg subcutaneously), and reported tolerance only in animals treated daily with haloperidol (1.5 mg/kg subcutaneously) and tested repeatedly on a horizontal bar. On the other hand, evidence of possible sensitization, that is an increase in catalepsy, was observed with various haloperidol doses, daily and weekly administration schedules, and testing with a horizontal bar and inclined screen (Barnes et al. 1990). Arguably, it will be important going forward to confirm or refute the present findings in a similar paradigm, that is using similar routes of administration, doses, and measures of catalepsy.

**Effect on Locomotor Activity**

Following up on this last point, administration schedule has, in fact, been found to influence haloperidol-induced hypolocomotion. Carey and colleagues (1984) demonstrated that animals with more frequent exposure to haloperidol (twice daily at 0.5 mg/kg IP) exhibited a trend towards recovery of spontaneous motor activity (i.e. tolerance), whereas intermittent haloperidol exposure (once every other day at 0.5 mg/kg intraperitoneally) led to a decrease in activity across time. There are other existing reports on the effects of transient versus continuous haloperidol administration on locomotion, although these studies were designed using sensitization models, where animals were either administered stimulants such as amphetamine (Samaha et al. 2008) and phencyclidine (Nsimba 2009;
Zhang et al. 2013) or tested after haloperidol withdrawal (Gianutsos et al. 1974; Clow et al. 1979; Meng et al. 1998; Samaha et al. 2007).

In our preliminary research (Preliminary Work I), a significant increase in locomotor activity (tolerance), as measured by total infrared beam breaks (all activity), beam lengths (cage crossings), and top plane beam breaks (rearing), was observed in animals continuously, but not intermittently, administered haloperidol. In contrast, animals that received intermittent haloperidol exposure via daily subcutaneous injections demonstrated a trend toward a significant decrease in locomotion between Week 1 and Week 3 of the study.

In Experiment 1, a paradigm was employed to track locomotor activity and measure it in terms of total distance travelled (in centimeters) and velocity (centimeters/second). To our knowledge, a chronic animal model of APD tolerance as measured by such changes has not been evaluated. Earlier preclinical work examining the relationship between these outcome measures and dopamine D₂ receptor antagonism examined either the acute effects of APDs (Starr et al. 1986) or used 6-hydroxydopamine lesions (Capper-Loup et al. 2013), which found suppression of ‘fast movements’ and a trend toward a significant decrease in mean velocity, respectively.

As previously discussed, in our paradigm (Experiment 1) treatment (continuous versus intermittent haloperidol exposure) did not produce any significant
between- or within-group effects with regard to locomotor activity, although there was a trend toward an increase in mean velocity in the haloperidol-continuous group. This said, the fact that tolerance was found in the remaining outcome measures (e.g. catalepsy, exploratory behaviour) argues against the possibility that the animals did not receive sufficient APD exposure.

**Effect on Exploratory Behaviour:**

Exploratory behaviour has been used as a learning and memory paradigm in animal research since the late 1980s (Bevins et al. 2002). This preclinical model capitalizes on novelty to examine behavioural changes (e.g. sniffing a novel object), thereby providing a means of examining innate exploratory behaviour (Antunes et al. 2012).

In Experiment 1, exploratory behaviour was included as an outcome measure based on evidence that it is mediated, at least in part, through dopamine. For instance, previous reports have established that dopamine blockade (e.g. dopamine denervation) decreases exploratory behaviour, while dopamine agonism (e.g. apomorphine) increases novel object seeking (Fink 1980; Alttoa et al. 2009). Further, elevated extracellular dopamine levels and D₂ receptors in the striatum have been observed in highly explorative animals (Alttoa et al. 2009). Based on this line of evidence, the present work investigated whether continuous versus intermittent antipsychotic exposure would alter exploratory activity; to our
knowledge, this question has not been systematically addressed in preclinical or clinical research. As noted, it was found that the total amount of time spent in a novel object-paired zone significantly increased across the course of the study in the haloperidol-continuous group. Conversely, the latency of entry to a novel object-paired zone did not produce a significant treatment or time x treatment effect, although there was a significant effect of time. Across sessions, latency of entry decreased in all groups, which almost reached significance in the haloperidol-continuous group. These findings do raise the question of whether these measures are equally representative of exploratory behaviour or, alternatively, whether there are different facets of exploration. One might argue, for instance, that time spent in a novel object-paired zone models exploration of a novel object more so than latency to first approach the object, which may be more easily influenced by anxiety, attention, and preference for novelty (Antunes et al. 2012). In most preclinical studies, exploration is defined as the orientation of an animal’s nose toward the novel object, which includes sniffing or touching with the nose (Antunes et al. 2012). In this sense, despite our inability to observe significant changes across time in terms of the latency of entry to the novel-object-paired zone, the significant increase in the total time spent in the novel object-paired zone, as observed in the haloperidol-continuous group, may still implicate tolerance following continuous but not intermittent APD exposure, as measured by at least one component of exploratory behaviour.
**Interpretation of Results:**

With the wide array of measures obtained in the present work, how can the various findings be tied together? As indicated in the Introduction, there are numerous theories that have been posited to explain drug tolerance. Given that research in the field of APD tolerance is limited, at best, one can only speculate about the potential mechanisms underlying the present findings. Various notions were reviewed at the outset, and several more are briefly discussed here for completeness.

Drug tolerance is not the development of tolerance to a *drug* per se, but tolerance to *an effect(s) of a drug* (Stewart et al. 1993); where not all drug effects are susceptible to tolerance (Meyer et al. 2005), and in those that are, the rate at which tolerance develops may differ. This would accommodate for the evidence of tolerance in some outcome measures (e.g. catalepsy, locomotor activity as seen in Preliminary Work I, total time spent in a novel object-paired zone), but not others (e.g. mean velocity, total distance travelled, latency of entry to novel object-paired zone). The exact mechanisms involved in ‘uneven’ development of tolerance remain a source of speculation, but it would not be unreasonable to imagine that the different facets of exploratory behavior, for example, are mediated by different mechanisms. Moreover, it is possible that the impact of biological and non-biological factors may vary as a function of the component being evaluated.
Also of possible relevance to the present study is the notion that sensitization and tolerance may occur simultaneously. Put simply, tolerance and sensitization are involved in maintaining adaptive responses to repeated changes in the internal or external environment (Celerier et al. 2001). Although these processes are often viewed as independent of one another, there is evidence to suggest that they may act concomitantly. For example, in addiction research it has been observed that tolerance to the analgesic and sedative effects of opiates can occur at the same time as sensitization toward activating effects of opiates such as locomotor hyperactivity (Stewart et al. 1993). This theory has been supported by earlier preclinical research in which opiates produced both analgesia and hyperalgesia (Celerier et al. 2001), suggesting that tolerance, to the analgesic effect of opiates could, at least in part, result from sensitization. Specifically in the context of our findings, while also considering earlier research that has found an increase in dopamine D₂ receptors following continuous haloperidol exposure (Samaha et al. 2008), it is possible in the present study that tolerance (e.g. decrease in catalepsy in haloperidol-continuous animals) and sensitization (e.g. increase in catalepsy in haloperidol-intermittent animals) were concomitantly observed.
Clinical Implications:

As noted, antipsychotics represent the cornerstone of treatment programs for individuals with schizophrenia and schizoaffective disorder. Moreover, they often are used routinely in the treatment of related disorders such as bipolar disorder, as well as in other diagnostic categories such as autism and Tourette's. Indeed, paralleling the development of the second-generation antipsychotics has been an exponential increase in antipsychotic use, including increasing off-label use of these medications (McKean & Monasterio 2012).

While much has changed about how we dose antipsychotics, this is really confined to how much. The technological advances of neuroimaging, and in particular PET, has afforded for the first time an in vivo means of establishing dosing based on D\textsubscript{2} occupancy, considered to be the sine qua non of antipsychotic efficacy (Kapur & Remington 2001).

What has received virtually no attention from the standpoint of dosing is how often these medications are administered. That these drugs need to be administered regularly to diminish risk of relapse is clearly acknowledged based on a strong body of evidence indicating increased risk of relapse in the face of antipsychotic discontinuation (Gitlin et al. 2001; Boonstra et al. 2011). Further to this point, antipsychotic nonadherence is a well-established risk factor in poorer

In the face of such risks, the field has held to the notion that continuous antipsychotic treatment is required to diminish risk of clinical deterioration. At the same time, though, there is a body of evidence, reviewed here, that indicates a) continuous antipsychotic response may not be required in order to maintain response once established, and b) continuous exposure may also be associated with diminished response (i.e. tolerance) that could compromise efficacy over time. Again, pursuit of options to continuous dosing have very likely been influenced by problems in general with adherence, in addition to concerns of relapse with any strategies that are less than continuous.

There may be reason to revisit this issue. At a clinical level this has, in fact, been explored although for somewhat different reasons. Concerns about the long term impact of these medications paved the way for efforts in the past to allow for gaps during maintenance antipsychotic treatment in the form of “drug holidays”; unfortunately, this strategy resulted in increased risk of relapse, although intervals off medications were often months in duration (Gaebel et al. 1994; Newton et al. 1989; Prien et al. 1973) However, more recent work, guided by PET findings indicating high and continuous antipsychotic dosing is not required to sustain response (Remington and Kapur 2011), has led to data indicating the fixed, finite intervals off medication (i.e. “extended” dosing) do not carry the same
increased risk of relapse (Remington 2005; 2011). Thus, there is now evidence that suggests psychosis can be controlled without continuous antipsychotic dosing, which also affords a model for examining the question of tolerance. In short, the question of tolerance takes on much more relevance if, in fact, there are alternative treatment strategies to continuous antipsychotic exposure that are tenable in the clinical setting.

Following this same line of thinking, it is also important to acknowledge that other potential benefits may be accrued through a strategy such as extended dosing. For example, alternate dosing affords a 50% reduction in antipsychotic exposure although, as of yet, the nature and extent of benefits that may be gained with such an approach have not been established. Ideally, the hope would be that a plethora of side effects would be reduced, side effects that span a spectrum from CNS (e.g. cognitive slowing, sedation) to metabolic (e.g. weight gain, diabetes) to motor (e.g. EPS, including TD). It may therefore be possible to enhance efficacy through decreased tolerance, while improving numerous burdensome side effects, but controlled trials would be required to verify these hypotheses.

Strengths, Limitations, and Future Directions:

Animal Models of Neuropsychiatric Illness

Animal models are not without limitations, and they have been challenged in the
context of illnesses such as schizophrenia, characterized as it is by features that simply cannot be mirrored in non-human models (e.g. hallucinations, paranoia). The present study, though, focused specifically on the issue of antipsychotic tolerance and employed simple behavioural measures that were chosen based on evidence indicating they are, at least in part, influenced by dopamine. Moreover, the model chosen here circumvented added strategies (e.g. sensitization) that, while useful from the standpoint of modeling schizophrenia, add additional levels of complexity to the interpretation of results. In addition, efforts were made to include behaviours with high predictive validity for APD-induced behavioural changes (e.g. catalepsy). Though our work does not model schizophrenia per se, it does model the use of antipsychotics clinically. Maintenance therapy, the cornerstone of psychiatric care, is currently defined as continuous antipsychotic exposure, which begs the question of possible tolerance in the face of prolonged exposure. Moreover, there is evidence from the clinical setting that in at least a subgroup of individuals being treated chronically with antipsychotics that attenuated response over time is observed. In this context, an animal model may be well suited to examine the induction of tolerance to continuous antipsychotic exposure, and both behavioural and biochemical outcome measures may prove beneficial in this regard.
Catalepsy

While catalepsy was considered an important measure because of its clear relationship to antipsychotics and dopamine D$_2$ blockade, its induction required ‘fine tuning’ here. After the first test day in Experiment 1, there were concerns that the level of catalepsy (i.e. latency to remove both forepaws from a fixed position), which was less than typically reported by other studies, could possibly limit our findings; namely in the haloperidol-intermittent group, there were concerns that catalepsy scores would be biased by a floor effect. For example, on Test Day 1, animals intermittently and continuously treated with haloperidol were cataleptic on average for 25.50 seconds and 97.39 seconds, respectively, whereas catalepsy scores ranging from 120-180 seconds are commonly reported in the literature. It is likely that our choice of dose (0.1 mg/kg subcutaneously; ~83% D$_2$ receptor occupancy, Turrone et al. 2003), which is notably lower than what is conventionally used when evaluating catalepsy (0.5-5.0 mg/kg subcutaneously; ~90% D$_2$ receptor occupancy, Kapur et al. 2003) may account for this. Our selection of a lower dose, and subsequently a lower occupancy level, was intentional and made in an effort to balance induction of catalepsy against other behavioural measures (locomotor and exploratory activity) that could be suppressed in the face of high levels of catalepsy. Fortunately, this did not occur as, interestingly, catalepsy progressively increased across time in animals exposed to intermittent treatment, whereas the opposite was found in animals with continuous haloperidol exposure. Going forward, it still may be of
interest to increase the haloperidol dose to determine if evidence of tolerance, as was found in the present study, can be intensified (e.g. a greater attenuation in catalepsy in haloperidol-continuous animals). Other work has supported this, indicating greater evidence of tolerance to haloperidol with higher doses (Carey et al. 1984), whereas daily administration of haloperidol at lower doses favoured sensitization or a heightened drug effect (Barnes et al. 1990). It remains that we actually know very little about the chronic effects of APDs on catalepsy, as existing research is typically confined to acute studies that are less than 1 week in duration (Campbell et al. 1981). It would be useful for future studies to examine the long-term effects of varying APD doses on catalepsy over a period greater than 3 weeks.

**Locomotor Activity**

In Preliminary Work I, total beam breaks (all activity), beam lengths (cage crossings), and top-plane beam breaks (rearing) were used as indicators of locomotor activity, whereas in Experiment 1, locomotion was measured by mean velocity and total distance travelled. Typically, when testing the motoric effects of drugs in animals, changes in motility are quantified in terms of total activity (e.g. distance travelled) (Starr et al. 1986). Arguably, such data do not adequately capture the nuances of the activity (Starr et al. 1986); as an example, it has been suggested that gross measurements of locomotion can give a misleading impression of activity level, as was found in an earlier study where total distance
travelled signified unchanged activity scores whereas animals visibly appeared hyperactive (Starr et al. 1986). In the present study the opposite was evident, as haloperidol treated animals (both continuous and intermittent) appeared hypoactive, yet total distance travelled was comparable to controls. Based on the findings of Starr and colleagues, locomotor velocity was also examined here, which provided a slightly better approximation of the animals’ activity level.

In working with antipsychotics in behavioural paradigms, it is important to at least consider sedation. Haloperidol’s motor inhibiting effect has been well established in both preclinical and clinical research; however, when examining drug-induced hypolocomotion it can be difficult to differentiate between motoric inhibition and sedation. To complicate matters further, sedation can be difficult to assess, although reports have indicated that it can successfully be quantified using a sedation rating scale (Salamone et al. 1996). Observations for the presence of behavioural indices of sedation, such as ptosis (eyelid drooping) prior to and following drug administration have shown to be effective when attempting to isolate motor impairments from sedation (Salamone et al. 1996).

In this particular study, it cannot be presumed that the decreased locomotion seen in our haloperidol-treated animals was entirely the result of motoric, and not sedative, drug effects. Of note though, Salamone and colleagues (1996) examined this issue in animals receiving haloperidol (0.05-0.15 mg/kg subcutaneously) at comparable doses to the present study (0.08, 0.10 mg/kg subcutaneously), and found little or no sedation. It remains that when examining
the behavioural effects of motor impairing drugs, it is useful to distinguish motor inhibition and sedation when evaluating locomotor activity. Other strategies include use of alternative paradigms such as conditioned avoidance responding (CAR).

Conclusions:

Continuous and prolonged antipsychotic exposure, currently representing the standard of care in illnesses like schizophrenia, by its very nature raises the question of tolerance. Here, a multi-tiered, comprehensive, behavioural approach was employed to compare the effects of continuous and intermittent haloperidol exposure in an animal model. Our findings are consistent with earlier preclinical reports that have implicated chronic and continuous antipsychotic therapy in reduced drug effects. Although tolerance was found in only some of our outcome measures, it is noteworthy that it is not uncommon for tolerance to develop to some of a drug’s effects and not others (Baldessarini et al. 1999). Perhaps the most relevant finding here was the changes suggestive of behavioural tolerance in catalepsy, which is arguably one of the better established measures of D₂ activity amongst those included here. Also of note is the fact that the intermittent dosing employed here now has a foothold in clinical practice with evidence that ‘extended dosing’, which offers transiently high D₂ occupancy within a framework of finite gaps, is a viable option that can be investigated without necessarily
increasing risk of relapse. This gives direct translational value to the type of preclinical work outlined here.

Going forward, we are in a position to translate results from such preclinical work to clinical studies that can further examine this issue in humans. If, in fact, the cumulative body of evidence supports tolerance, maintenance antipsychotic therapy, considered critical to favourable long-term outcomes, can be modified in accordance with these findings.
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