A ROLE FOR NEUROPEPTIDES IN TARDIVE DYSKINESIA: BLOCKADE OF OPIOID AND NON-OPIOID PEPTIDE RECEPTORS SUPPRESSES NEUROLEPTIC-INDUCED VACUOUS CHEWING MOVEMENTS

by

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ABSTRACT

The treatment of schizophrenia with chronic neuroleptic therapy leads to the development of tardive dyskinesia, an involuntary movement disorder principally manifest in the orobuccal region, in approximately 20-30% of patients. While the pathogenesis of tardive dyskinesia remains uncertain, altered function of both opioid and non-opioid peptides in the basal ganglia has been implicated in its emergence. The levels of both the opioid peptides dynorphin and enkephalin are increased following long-term treatment with classical antipsychotic drugs. In addition, the levels of striatal neurotensin, a non-opioid peptide, are augmented following chronic neuroleptic treatment. Using a rodent model of tardive dyskinesia, vacuous chewing movements induced by chronic neuroleptic therapy, we have examined the role of these opioid and non-opioid peptides in the emergence of tardive dyskinesia. Animals received chronic administration of fluphenazine decanoate (25mg/kg i.m.) or its vehicle, sesame oil, every 3 weeks for a minimum of 18 weeks. Animals were then surgically implanted with stainless steel guide cannulae aimed at the striatum, the substantia nigra pars reticulata, or the globus pallidus. In order to examine the role of opioid peptides in tardive dyskinesia, we infused the kappa opioid receptor antagonist, nor-binaltorphimine, into the substantia nigra pars reticulata, or the non-specific opioid antagonist, naloxone, into the globus pallidus. To further clarify the specific receptor subtypes involved, we infused D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr Amide (CTOP), a selective mu-opioid receptor antagonist, or naltrindole, a specific delta-opioid receptor antagonist, into the globus pallidus of separate groups of animals. For investigation of the role of the non-opioid peptide neurotensin in tardive dyskinesia, we infused the neurotensin antagonist 2-[(1-(7-chloro-4-quinolinyl)-5-(2,6-dimethoxyphenyl)pyrazol-3-yl)carbonylamino]tricyclo(3.3.1.1.3.7) decan-2-carboxylic acid (SR48692) into the striatum, the substantia nigra pars reticulata, or the globus pallidus. Chronic treatment with fluphenazine decanoate resulted in the robust development of vacuous chewing movements. All antagonist compounds suppressed vacuous chewing movements to varying degrees, depending on the compound used and the location of infusion. These results suggest a possible role for opioid and non-opioid peptides in the emergence of tardive dyskinesia. Thus, drug treatments which reduce or prevent the effects of increased expression of these peptides may be helpful in the management of tardive dyskinesia.
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ANOVA, analysis of variance; AP, anteroposterior; CO-I, cytochrome oxidase I; CTOP, D-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr Amide; δ, delta; DBS, deep brain stimulation; DMSO, dimethyl sulfoxide; DV, dorsoventral; DYN, dynorphin; ENK, enkephalin; FLU, fluphenazine decanoate; GABA, γ-amino-butyric acid; GAD, glutamic acid decarboxylase; GP, globus pallidus; GPe, external globus pallidus; GPi, internal globus pallidus; 6-OHDA, 6-hydroxydopamine; ICV, intracerebroventricular; κ, kappa; L-DOPA, levodopa; LID; levodopa-induced dyskinesia; ML, mediolateral; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; μ, mu; NAcc, nucleus accumbens; NAL, naloxone; NTI, naltrindole; nor-BNI, nor-binaltorphimine; NT, neurotensin; NTS1, NT receptor 1; NTS2, NT receptor 2; PPE-A, preproenkephalin-A; PPE-B, preproenkephalin-B; PPN, pedunculopontine nucleus; RB101, N-[(R,S)-2-benzyl-3[(S)(2-amino-4-methylthio)butyl dithio]-1-oxopropyl]-L-phenylalanine benzyl ester; SC, superior colliculus; SNK, Student Newman Keuls; SNr, substantia nigra pars reticulata; SP, substance P; SR48692, 2-[(1-(7-chloro-4-quinolinyl)-5-(2,6-dimethoxyphenyl)pyrazol-3-yl)carbonylamino]tricyclo(3.3.1.3.7)decan-2-carboxylic acid; STN, subthalamic nucleus; THAL, Thalamus; TD, tardive dyskinesia; VCMs, vacuous chewing movements; VEH, vehicle.
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CHAPTER I The Basal Ganglia and Tardive Dyskinesia

1.1 Introduction

While abnormal involuntary movements can develop throughout the course of a degenerative disorder as a result of profound changes in neuroanatomical structure and function (e.g. Huntington's disease), dyskinesia can also be an iatrogenic effect resulting from drug therapies used to manage the disorder (e.g. Parkinson's disease and schizophrenia). Dyskinesias are characterized by the development of involuntary movements, which upon emergence may persist following withdrawal from drug treatment. The chronic treatment of schizophrenia with typical antipsychotics leads to the development of tardive dyskinesia (TD), a disorder typically characterized by involuntary movements of the orobuccal region, in approximately 20-30% of patients (Kane & Smith, 1982; Yassa & Jeste, 1992). While atypical antipsychotic treatments are less likely to result in the development of extra-pyramidal side effects, haloperidol, a typical neuroleptic, has been, until recently, the most commonly used clinical treatment for schizophrenia (Kapur et al., 2000). Furthermore, once treated with typical antipsychotics, drug withdrawal and subsequent change to atypical antipsychotic therapy may be unable to ameliorate TD (Modestin et al., 2000). Thus, it remains crucial to examine the mechanisms involved in the emergence of TD by typical antipsychotics in order to provide means by which to counteract the development of this drug-induced disorder.

While the precise mechanisms contributing to the development of TD remain elusive, it has been suggested that alterations in the balance of activity between the direct striatonigral and the indirect striatopallidal output projections of the basal ganglia may lead to the emergence of dyskinesia (Trugman et al., 1996; Vitek & Giroux, 2000). Thus, an interruption of this abnormal activity in either pathway may result in the attenuation of TD. In order to study the mechanisms involved in the development of TD, a rodent model, vacuous chewing movements (VCMs)
induced by chronic neuroleptic therapy, has been developed and widely used (Iversen et al., 1980; Waddington et al., 1983; Stoessl et al., 1989, 1993; Glenthoj et al., 1990; Tamminga et al., 1990; Diana et al., 1992; Egan et al., 1994; Andreassen & Jorgensen, 1995).

1.2 The Basal Ganglia

1.2.1 Nuclei of the Basal Ganglia

The basal ganglia consist of a number of subcortical structures so named for their location in the deep-lying layers of the forebrain (Wilson, 1928). There are five nuclei central to the configuration of the basal ganglia. They are the caudate nucleus, the putamen, the globus pallidus (in combination with the putamen also referred to as the lenticular nucleus), the substantia nigra and the subthalamic nucleus.

Initially, the basal ganglia were conceptualized as a funnel for integrating the various inputs of the cerebral cortex and sending this information to the motor cortex by way of the ventrolateral thalamus (Evarts & Thach, 1969; Kemp & Powell, 1971; Allen & Tsukahara, 1974; Alexander et al., 1986). Thus, association areas of the cerebral cortex could influence the motor cortex via the basal ganglia. However, as research has provided insight into the complexity of the segregated and parallel inputs to the basal ganglia as well as the diverse regions of the cortex affected by its nuclei, this funnel conceptualization can now be viewed as overly simplistic (Alexander et al., 1986). Thus, the circuitry of the basal ganglia is now regarded as a complex system whose influence extends to various nuclei of the thalamus and the cortex (Goldman-Rakic & Selemon, 1990).

1.2.2 Organizational divisions of the basal ganglia

The functional and anatomical organization of the basal ganglia has been divided into five subdivisions: the motor, oculomotor, dorsolateral prefrontal, lateral orbitofrontal, and anterior cingulate circuits (Alexander et al., 1986). These circuits are involved in a variety of
higher order functions and abnormal activity within these circuits occurs in neurodegenerative disorders such as Parkinson's and Huntington's disease as well as in psychiatric illnesses such as schizophrenia.

Fibers from the entire cerebral cortex contribute to the corticostriate projection, the input of primary significance to basal ganglia function. This corticostriate pathway conveys information from the motor, sensory, association and limbic areas. Each of these areas projects to a specific region of the striatum (the dorsal region of the caudate nucleus and putamen, also known as the neostriatum) and as such is involved in different behavioural functions. The putamen is largely involved in motor control, the caudate nucleus is involved with eye movements and cognition, and the ventral striatum (nucleus accumbens, the ventromedial caudate-putamen and the olfactory tubercle) is involved in emotional aspects of behaviour.

Specifically, the sensorimotor striatum corresponds to the dorsolateral area of the post-commissural putamen, the dorsolateral rim of the pre-commissural putamen and the lateral area of the caudate nucleus. This area of the striatum receives afferents from the motor, premotor, supplementary and cingulate cortical areas and the somatosensory cortex (Kunzle, 1975, 1977, 1978; Jones et al., 1977; Weisendanger & Weisendanger, 1984; Malach & Graybiel, 1986; Flaherty & Graybiel, 1993; Nakano et al., 1995, 2000; Takada et al., 1998a; 1998b; Inase et al., 1999). Once the information from these various inputs is processed, the motor circuit, which is implicated in both the preparation for and the achievement of movement (Alexander & Crutcher, 1990; Albin et al., 1989), primarily projects back to the supplementary motor area (Alexander et al., 1986). Other investigators, however, have found projections back to more than one cortical area (Nakano et al., 2000).

The associative striatum corresponds to the head, the mid-section, and the tail of the caudate nucleus, as well as the pre-commissural putamen and the ventromedial post-commissural
putamen. Its cortical afferents are from the prefrontal, temporal, and posterior parietal and preoccipital cortices as well as the frontal and supplementary eye fields (Goldman & Nauta, 1977; Kunzle & Akert, 1977; Kunzle, 1978; Goldman-Rakic et al., 1984; Percheron et al., 1984; Selemon & Goldman-Rakic, 1985, 1988; Stanton et al., 1988; Saint-Cyr et al., 1990; Yeterian & Pandya, 1991, 1993, 1995, 1998; Parthasarathy et al., 1992; Cheng et al., 1997; Nakano et al., 2000). Upon completion of the associative circuit through the basal ganglia, the efferents project via the ventral anterior and mediodorsal thalamic nuclei to such associative cortical areas as the dorsolateral prefrontal and lateral orbitofrontal areas (Alexander et al., 1986; Nakano et al., 2000).

The limbic striatum, also known as the ventral striatum, extends from the dorsomedial to the ventral caudate nucleus, the nucleus accumbens and the ventromedial pre-commissural putamen as well as the medium-sized cell area of the olfactory tubercle (Alexander et al., 1986). The afferents innervating the limbic striatum project from the piriform, prelimbic and infralimbic cortices, the hippocampus, and the amygdala (Yeterian & Van Hoesen, 1978; Van Hoesen et al., 1981; Selemon & Goldman-Rakic, 1985; Yeterian & Pandya, 1991; Kunishio & Haber, 1994; Eblen & Graybiel, 1995; Haber et al., 1995; Augustine, 1996; Cheng et al., 1997; Nakano et al., 2000). This limbic circuit projects back to such limbic cortical areas as the medial orbital, orbitofrontal and anterior cingulate areas (Nakano et al., 2000).

In addition to the primary corticostriatal input, the striatum also receives afferents from a number of other nuclei. The substantia nigra pars compacta and the retrorubral region project to the dorsal striatum and the ventral tegmental area projects to the ventral striatum (Jimenez-Castellanos & Graybiel, 1989; Nakano et al., 2000). As well, there is an input from the intralaminar nuclei of the thalamus to the neostriatum. The fibers from the centromedian nucleus of the thalamus, which receives efferents from the motor cortex, terminate in the putamen.
The basal ganglia output projections arise from the internal portion of the globus pallidus and the substantia nigra pars reticulata. These nuclei project to the thalamus, specifically, to the ventrolateral, the ventroanterior, and the mediodorsal nuclei of the thalamus. The internal globus pallidus has an additional projection to the centromedian nucleus of the thalamus. These thalamic projections lead to the prefrontal, premotor, and motor cortices as well as the supplementary motor area. In addition, the substantia nigra pars reticulata projects to the superior colliculus and pedunculopontine nucleus.

**1.2.3 The motor circuit of the basal ganglia**

The striatum, consisting of the caudate nucleus and the putamen in its dorsal division, receives afferents from the cerebral cortex as well as from the substantia nigra pars compacta. From the striatum, there are two output projections: the direct striatonigral pathway and the indirect striatopallidal pathway. The direct striatonigral pathway leaves the striatum and flows directly to the internal globus pallidus (GPi or entopeduncular nucleus in the rat) and the substantia nigra pars reticulata (SNr), which in turn project to the motor thalamus. The indirect striatopallidal pathway sends efferents to the external globus pallidus (GPe or globus pallidus (GP) in the rat) which project to the subthalamic nucleus (STN), then to the GPi/SNr, and from here to the motor thalamus. There is also an internal loop within this pathway by which the STN projects back to the GPe as well as a projection from the GPe to the GPi/SNr, which by-passes the STN altogether.

The control and initiation of movement are regulated by the balanced functioning of the basal ganglia’s motor circuit (Fig.1). The motor areas of the cortex project to the postero-lateral
Fig. 1. The circuitry of the basal ganglia in primates. Inhibitory projections are shown as black arrows, excitatory projections as gray arrows. Cortical information that reaches the striatum is conveyed to the basal ganglia output nuclei (GPi/SNr) via the direct and indirect pathways. The direct pathway is an inhibitory projection from the striatum to the GPi/SNr, the indirect pathway is comprised of inhibitory projections from the striatum to the GPe and from the GPe to the STN and an excitatory projection from the STN to the GPi/SNr. This information is then projected back to the cerebral cortex via a relay in the thalamus or conveyed to various brainstem structures. The dopaminergic neurons of the SNc exert a net excitatory effect on spiny neurons giving rise to the direct pathway by activation of dopamine D1 receptors, whereas they exert a net inhibitory effect on spiny neurons giving rise to the indirect pathway by activation of dopamine D2 receptors. Cortical information can also reach the basal ganglia via the corticostriatal projection. GPe, external globus pallidus; GPi, internal globus pallidus; PPN, pedunculopontine nucleus; SC, superior colliculus; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; THAL, thalamus. (Adapted from Smith et al., 1998).
putamen. In the putamen, these excitatory glutamatergic inputs make synaptic connections with inhibitory medium spiny neurons expressing γ-amino-butyric acid (GABA). The neurons of the direct striatonigral pathway bearing dopamine D1 receptors and co-expressing GABA, substance P (SP), and dynorphin (DYN) project directly to the GPi/SNr where they provide an inhibitory influence (Obeso et al., 2000). The indirect striatopallidal pathway connects indirectly to the GPi/SNr via the GPe and the STN (Obeso et al., 2000). The neurons of the indirect pathway projecting to the GPe are GABAergic neurons, which co-express the peptide enkephalin (ENK) and bear dopamine D2 receptors. The efferents from the GPe to the STN are inhibitory GABAergic efferents. The STN contains glutamatergic neurons; hence the effect of these neurons on the GPi/SNr is excitatory. Consequently, when the indirect striatopallidal pathway is stimulated, the result is inhibition of the GPe, disinhibition of the STN and excitation of the GPi/SNr. Thus, under the influence of both the direct striatonigral and the indirect striatopallidal pathways, the GPi/SNr output nuclei are influenced by opposing inhibitory and excitatory effects. These opposing effects modulate the activity of brainstem and thalamo-cortical neurons thereby influencing motor activity. Therefore, the balance of activity in the direct striatonigral and indirect striatopallidal pathways is crucial to the normal execution of motor behaviours (Obeso et al., 2000).

1.2.4 Motor circuit functioning in hypokinetic disorders

According to the classical model, the net result of the pathophysiology of such hypokinetic disorders as Parkinson's disease is increased activity of the basal ganglia's output nuclei resulting in excessive inhibition of the brainstem and thalamo-cortical motor systems (Obeso et al., 2000). The loss of the dopaminergic nigral afferents to the neostriatum in Parkinson's disease results in decreased excitation of the neurons of the direct striatonigral pathway leading to a reduction of inhibitory influence on the GPi/SNr. In contrast, reduced
inhibition of the indirect striatopallidal pathway resulting from dopamine deficiency leads to over-inhibition of the GPe, disinhibition of the STN, and increased excitation of the GPi/SNr neurons. The hyperactivity of GPi/SNr neurons increases inhibition of the thalamic nuclei resulting in a movement deficit.

1.2.5 Motor circuit functioning in hyperkinetic disorders

The classical model proposes that the dyskinetic movements found in Huntington's chorea, levodopa-induced dyskinesia, and neuroleptic-induced TD are the result of reduced activity in the STN and the GPi/SNr. Excessive inhibition of the indirect striatopallidal pathway leads to disinhibition of the GPe, over-inhibition of the STN, and reduced excitatory drive to the GPi/SNr (Obeso et al., 2000). This produces hypoactivity in the GPi/SNr neurons reducing their inhibitory influence on the thalamo-cortical neurons and thereby producing an accompanying excess of excitation to the cortical motor areas. The net result of this excessive cortical excitation is the appearance of dyskinesia.

1.2.6 Evidence for and against the classical model of basal ganglia motor functions

While the classical model ultimately fails to account for the complexities of the motor system, there is a body of evidence that supports a number of predictions made by the model. For example, in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys exhibiting the symptoms of parkinsonism, increased levels of mRNA encoding for cytochrome oxidase I (CO-I), an enzyme of the respiratory mitochondrial chain involved in cellular metabolism, are found, with in situ hybridization, in the STN, the GPi, and the SNr (Vila et al., 1997). In situ hybridization also reveals increased levels of mRNA encoding for glutamic acid decarboxylase (GAD), the enzyme crucial to the synthesis of GABA from glutamate, in the GPi and the SNr of MPTP-treated monkeys (Herrero et al., 1996; Vila et al., 1996; Obeso et al., 2000). Furthermore, in MPTP monkeys the firing frequency of neurons is higher than normal in
both the STN and GPi and reduced in the GPe (Bergman et al., 1994; Filion et al., 1991; Obeso et al., 2000), in accordance with a state of reduced inhibition from the GPe to the STN and increased excitation of the GPi.

When MPTP monkeys are treated with levodopa (L-DOPA) or apomorphine, a direct dopamine agonist, not only do they show improved motor function but also reduced firing frequency of neurons in both the STN and GPi (Papa et al., 2000; Obeso et al., 2000) and increased neuronal activity in the GPe (Boraud et al., 1998; Obeso et al., 2000). This is also the case in 6-hydroxydopamine (6-OHDA)-lesioned rats (Papa et al., 2000). In addition, the improved motor function in L-DOPA treated MPTP monkeys is accompanied by a reduction in CO-I and GAD mRNA levels in the STN, GPi, and SNr (Herrero et al., 1996; Vila et al., 1996, 1997; Obeso et al., 2000), indicating reduced hyperactivity in these nuclei. Furthermore, pallidotomy and deep-brain stimulation (DBS), which simulates the effects of a lesion without destroying brain tissue, of the STN or GPi in parkinsonians often induces a marked clinical improvement (Brown et al., 1999). Finally, as predicted by the model, electrophysiological and positron emission tomography studies indicate that pallidotomy and DBS also normalize patterns of cerebral blood flow in cortical motor areas that are hypocative in Parkinson’s disease (Limousine et al., 1997; Obeso et al., 2000).

However, the same studies, which found increased levels of CO-I and GAD mRNAs in the STN and GPi/SNr, do not report reduced mRNA levels in the GPe in either MPTP monkeys or rats lesioned with 6-OHDA (Herrero et al., 1996; Vila et al., 1997; Obeso et al., 2000). As well, in the GPe of MPTP monkeys with levodopa-induced dyskinesia (LID), mRNA levels for both CO-I and GAD are not increased compared with the parkinsonian state (Herrero et al., 1996; Vila et al., 1997; Obeso et al., 2000). Furthermore, there is evidence to suggest that lesioning of the GPe in MPTP monkeys may exacerbate rather than ameliorate LID (Blanchet et
al., 1994). These findings contradict the model, which supposes that, in LID, disinhibition of the GPe leads to hypoactivity in the GPi/SNr neurons producing a concomitant excess of excitation to the cortical motor areas.

The classical model proposes opposing mechanisms for hyperkinetic and hypokinetic disorders suggesting that TD may be the result of an excess of dopaminergic stimulation and drug-induced parkinsonism the result of reduced dopaminergic activity. As such, these hypotheses would preclude the concomitant existence of TD and neuroleptic-induced parkinsonism. However, a number of studies have found that TD and neuroleptic-induced parkinsonism can co-exist (Richardson et al., 1982; Wirshing et al., 1989; Hansen et al., 1991; Saltz et al. 1991; McCreadie et al., 1992). These findings suggest that a different explanation for the pathophysiology of these disorders is necessary in order to account for their co-occurrence.

Perhaps the largest and most critical problem encountered by the classical model is the effect of pallidotomy. Pallidotomy eliminates LID in parkinsonian patients, ameliorates hemiballismus (Marsden & Obeso, 1994; Obeso et al., 2000) and improves TD (Wang et al., 1997). Furthermore, pallidotomy, thalamotomy or DBS of the STN do not impair voluntary movement as predicted by the model (Marsden & Obeso, 1994).

The experimental and clinical findings have necessarily led to a modification of the classical model. The emphasis is now on the balance and pattern of activity in the motor circuit of the basal ganglia and how alterations of these may yield the deficits found in diseased states. Thus, disorders like dyskinesia may be due to altered activity in both the direct striatonigral and the indirect striatopallidal pathways concurrently. This hypothesis is supported by the finding that, in both rats and monkeys, numerous striatal projection neurons have highly collateralized axons which branch to two or three striatal targets (Kawaguchi et al., 1990; Smith & Bolam,
1991; Bolam & Smith, 1992; Parent et al., 1995; Wu et al., 2000). Therefore, any abnormality in striatal function would likely contribute to changes in activity in both pathways.

1.3 Tardive Dyskinesia

1.3.1 The pathophysiology of tardive dyskinesia

While a number of hypotheses have been advanced to elucidate the pathophysiology of TD, none of them can satisfactorily explain all features of the disorder. Thus, to date, there is no truly effective treatment for TD, though a higher dose of neuroleptic may be successful in suppressing TD (Bateman et al., 1979; Fann et al., 1982; Jeste et al., 1988). Another strategy is discontinuation of neuroleptic therapy. However, discontinuation of treatment will likely result in psychiatric relapse, may uncover latent TD, and ultimately may be unsuccessful in ameliorating TD once it is established.

As typical antipsychotics selectively antagonize the dopamine D2 receptor, it has been suggested that chronic dopamine D2 receptor blockade results in supersensitivity of the dopamine D2 receptor leading to a pathological condition. A significant criticism of this hypothesis can be found with regard to the timing of dopamine receptor up-regulation and the emergence of TD. The up-regulation of striatal dopamine receptors occurs rapidly after initiation of neuroleptic treatment, while the emergence of TD typically takes months, if not years (Christensen et al., 1976; Dewey & Fibiger, 1983; Fibiger & Lloyd, 1984). If dopamine receptor sensitivity were the substrate underlying TD, then the condition may be expected to emerge within weeks of treatment. Furthermore, while upregulation of dopamine receptors would self-correct following neuroleptic withdrawal, TD persists long-term or irrevocably. As well, the post-mortem tissue and PET studies of schizophrenics have failed to provide any consistent relationship between dopamine D2 receptor upregulation and TD (Casey & Gerlach, 1986; Kornhuber et al., 1989; Waddington, 1989; Blin et al., 1989).
The depolarization block hypothesis addresses some of the temporal discrepancies implicit in the dopamine receptor supersensitivity hypothesis. This model proposes that while acute administration of typical neuroleptics increase the activation of dopamine neurons in the substantia nigra, chronic administration of these drugs drastically reduces the number of spontaneously active dopamine neurons (Bunney and Grace, 1978; Grace and Bunney, 1986). This deferred inactivation of the midbrain dopamine neurons is said to correspond to the delayed development of both the therapeutic effects of antipsychotics as well as the emergence of extrapyramidal side effects (Bunney and Grace, 1978; Grace and Bunney, 1986). Thus, this hypothesis proposes that the therapeutic action of antipsychotic drugs is related to inactivation of dopamine neurons in the ventral tegmental area, while the emergence of motor side effects is connected to inactivation of dopamine neurons in the substantia nigra (Bunney and Grace, 1978; Grace and Bunney, 1986). More recent results, however, have failed to show evidence of depolarization block after chronic neuroleptic administration (Mereu et al., 1994, 1995). As well, while the model proposes that activation of excitatory inputs on dopamine neurons will be unable to enhance dopamine release (Grace, 1992), Klitenick and colleagues (1996) found that this was not the case. Their results suggested that mild stress produced by tail pinch and electrical stimulation of the prefrontal cortex were able to increase extracellular dopamine concentrations (Klitenick et al., 1996).

The GABA hypothesis of tardive dyskinesia was first suggested by Fibiger and Lloyd (1984). A number of studies have revealed selective loss of GABA-expressing striatal neurons in rodents following chronic exposure to neuroleptics (Pakkenberg et al. 1973; Nielsen & Lyon, 1978). In particular, the neurons of the ventrolateral striatum suffer the most loss, which in respect to tardive dyskinesia is particularly interesting as this area of the striatum is responsible for the control of oral musculature (Mackay, 1982). As predicted by the model, the loss of these
neurons should result in decreased GAD activity in the GP and the SNr. This prediction has been supported in studies conducted in both monkeys and rodents (Gunne & Haggstrom, 1983; Gunne et al., 1984; Johansson et al., 1990). As well, GAD concentration is reduced in the cerebrospinal fluid of schizophrenic patients with TD (Thaker et al., 1987). Furthermore, GABA agonist treatment has been found to attenuate vacuous chewing movements in rats treated with chronic neuroleptics (Kaneda et al., 1992; Gao et al., 1994) and to improve dyskinetic symptoms in schizophrenic patients with TD (Tamminga et al., 1979; Thaker et al., 1987; Cassady et al., 1992).

The excitotoxicity hypothesis was first proposed in connection with Huntington’s disease (Coyle & Schwarcz, 1976; McGeer & McGeer, 1976). Increased glutamate release due to chronic neuroleptic treatment has been found (Moghaddam & Bunney, 1983; Yamamoto & Cooperman, 1994; See & Chapman, 1994; See & Lynch, 1995) and thus suggests the possibility of excitotoxicity. However, the exact mechanisms whereby excitotoxicity would result in the emergence of TD remains unclear.

The most recent candidate for a role in the pathophysiology of TD is oxidative stress. As neuroleptics are shown to induce an increase in dopamine turnover, it has been suggested that this may lead to formation of reactive oxygen species (See, 1991). A number of groups have proposed that lipid peroxidation of the neuronal membrane by hydroxyl radicals may play a role in the emergence of TD (Cadet et al., 1986; Yokoyama et al, 1994). Furthermore, haloperidol has been shown to induce oxidative stress in vitro (Behl et al., 1996; Sagara et al., 1998; Post et al., 1998) and a toxic metabolite of haloperidol has been found, which has similar properties to the toxic metabolite of MPTP (Bloomquist et al., 1994; Fang et al., 1995). However, such toxic metabolites for other neuroleptics, with the same propensity as haloperidol to lead to TD, have not been found (Andreassen & Jorgensen, 2000).
While each of the hypotheses discussed has limitations, the proposal of a discrete mechanism in the emergence of TD may be the reason for its limited scope and success. Perhaps a number of the mechanisms suggested are working together to produce TD, as the complexity and inter-connectedness of the system involved makes a singular mechanism unlikely.

1.3.2 A rodent model of tardive dyskinesia

The development of a rodent model of TD has proven to be challenging and, to date, the existing models are not without their detractors. In fact, the rodent model of TD used in these experiments, vacuous chewing movements (VCMs) induced by chronic neuroleptic therapy, remains controversial.

Some criticisms of the neuroleptic-induced VCMs model are that VCMs are more analogous to Parkinsonian tremor (Salamone et al., 1990; Jicha and Salamone, 1991; Cousins et al., 1998) or acute dystonia (Rupniak et al., 1985, 1986) than to TD. These criticisms stem from the contrasting ways in which humans with TD and rats with neuroleptic-induced VCMs respond to anticholinergic treatment and to cholinomimetic drugs. While cholinomimetic agents tend to reduce TD in humans, anticholinergic treatment tends to exacerbate the symptoms of TD while ameliorating neuroleptic-induced parkinsonism (Fann et al., 1974; Klawans & Rubovits, 1974; Davis et al., 1976; Gelenberg et al., 1979; Stahl et al., 1982). The opposite findings have been reported with neuroleptic-induced VCMs in rats, whereby anticholinergic treatment suppresses VCMs and cholinomimetic drugs like physostigmine exacerbate them (Yamada & Furukawa, 1980; Gunne et al., 1982, Gunne & Haggstrom, 1983; Rupniak et al., 1983, 1985; Salamone et al., 1986, 1990; Kelley et al., 1989). However, the human studies have been conducted on small numbers of patients and have lacked adequate control for experimenter expectation. As well, the doses of cholinomimetics used in the rat studies were supramaximal for eliciting mouth movements (Gunne et al., 1982; Rupniak et al., 1983), while the high dose of anticholinergic
used by Rupniak and colleagues (1983) has been shown to elicit hyperactivity (Stoessl et al., 1989). Thus, while the anticholinergic treatment suppressed VCMs, this may be due to behavioural response competition rather than to a specific effect of anticholinergic drug treatment (Levy et al., 1987; Stoessl et al., 1989). Furthermore, in completely randomized double-blind studies, it appears that anticholinergic drugs may ameliorate TD in some humans (Lieberman et al., 1988; Wirshing et al., 1989). Moreover, others groups have been unable to alter neuroleptic-induced VCMs with the anticholinergics procyclidine (Waddington et al., 1986) and scopolamine or with the cholinomimetic physostigmine (Stoessl et al., 1989).

The time required for development of neuroleptic-induced VCMs has also raised some controversy as some studies have reported the emergence of VCMs upon the initiation of neuroleptic treatment (Rupniak et al., 1985, 1986). This is in contrast to TD in humans, which has a delayed onset. It has been argued, however, that acute and tardive VCMs have different pharmacological and neurochemical profiles (Egan et al., 1996). Importantly, the profile of tardive VCMs shares similarities with that of TD in humans: suppression by higher dose of neuroleptic, increased dynorphin mRNA and increased enkephalin mRNA (Egan et al., 1996). In addition, while acute administration of the neuroleptic haloperidol may induce oral movements in rats, it has a strong effect on behaviour overall at this early stage (Andreassen & Jorgensen, 1995). In contrast, chronic haloperidol treatment induces VCMs without substantially altering other types of behaviour (Andreassen & Jorgensen, 1995).

On the whole, though, most studies have reported the emergence of neuroleptic-induced VCMs after 8-15 weeks of treatment (Waddington, 1990). This time-frame is the rat equivalent of several years in man (Tamminga et al., 1990), thereby suggesting a strong temporal similarity to the emergence of TD. This, along with a number of other similarities, supports its use as a putative model of TD. Like TD, VCMs persist following withdrawal from medication.
(Waddington et al., 1983; Ellison, 1991). Likewise, they are suppressed by treatment with dopamine receptor antagonists (Stoessl et al., 1989) and by dopamine depletion with both reserpine and α-methyl-p-tyrosine (Diana et al., 1992).

As far as the other animal models of TD are concerned, more differences exist between them and TD in humans. The first of these other models examines the behavioural sensitization (i.e. oral stereotypies) to dopamine agonists in animals treated with chronic neuroleptics (Tarsy & Baldessarini, 1973; Clow et al., 1979; Pollock & Kornetsky, 1991; Pucilowski & Eichelman 1991). The crucial problem with this model is that, in humans undergoing chronic neuroleptic treatment, the oral stereotypies of TD develop spontaneously while this paradigm requires supplementary pharmacological manipulation. The second model examines ‘movelets’ of the mouth with specialized equipment (Ellison 1991). This model shares a number of similarities with TD in both the time required for the development of the ‘movelets’ and in their response to medication (Ellison 1991). However, this model requires that animals be restrained in a tube during observation, a situation which produces stress in the animals. As studies have suggested that stress may unnaturally elevate VCMs in control animals (Gunne et al., 1986; Glenthoj et al., 1990), this may reduce the reliability of observations made about drug effects on VCMs in this model.

Taking these issues into consideration, we believe that the model of VCMs induced by chronic neuroleptics is appropriate for investigating the potential role of altered peptidergic transmission in the pathogenesis of TD. This is supported by a recent literature review that explored the various animal models that have been employed (Turrone et al., 2002). In the current thesis, this model has been used to investigate the role of opioid peptides and neurotensin.
CHAPTER II Methodology

2.1 Animals and Surgery

The male Sprague Dawley rats (Animal Care Centre Breeding Unit, South Campus, UBC) used in each experiment weighed between 250 – 300 g. at the start of treatment. They were housed in pairs in Plexiglas shoebox cages in a temperature-controlled environment with a 12 h light/dark cycle (lights on at 0700 h). The animals had *ad libitum* access to water but were food restricted (45g/d) to minimize excessive weight gain during the chronic neuroleptic treatment period. The animals received a minimum of 7 injections (every 3 weeks for 18 weeks) of fluphenazine decanoate (FLU, 25 mg/kg, i.m.) or its vehicle (VEH, 1ml/kg, i.m.), sesame oil, before experimental treatment. Prior to all surgical procedures, the animals were pretreated with diazepam (2.5 mg/kg, i.m.), anaesthetized with 0.1% halothane gas, and placed in a Kopf stereotaxic frame. They were implanted with bilateral stainless steel guide cannulae, which were secured to the skull with jewelers' screws and acrylic dental cement. The coordinates used for cannulae placements were derived from Paxinos and Watson (1986). Stainless steel obturators were inserted in the cannulae in order to maintain their patency. Following surgery, a recovery period of a week was allowed before the behavioural testing began. Animals were treated in accordance with Canadian Council on Animal Care guidelines. All efforts were made to minimize animal suffering and the number of animals used was kept to a minimum.

2.2 Materials

Opioid (*nor*-binaltorphimine, naloxone, CTOP, and naltrindole) and non-opioid (SR48692) receptor antagonists were used for these experiments. Each drug was dissolved in vehicle just prior to testing each day in order to ensure drug potency. After preparation, each antagonist was injected into infusion cannulae and, immediately prior to behavioural testing, infused into the target region.
2.3 Behavioural Testing

On the test day, animals were placed individually in Plexiglas boxes (50 x 50 x 30 cm) for an habituation period of 1 hour. Following habituation, the animals were observed continuously for three minutes out of every six minute block, alternating between two animals, for a total of 10 blocks (60 min) each. The frequency and duration of various behavioural responses were recorded using a microcomputer keyboard and custom-designed software (BEBOP: Dr. M. Martin-Iverson, University of Western Australia). For each response of interest, a coded key was pressed at the onset and offset of each behaviour. After testing the animals were returned to their home cages.

The following behavioural responses were recorded: VCMs, grooming, locomotion, sniffing, and rearing. VCMs were defined as all undirected mouth movements including chewing and tongue protrusions while excluding jaw tremor and directed movements such as licking, eating, grooming, and yawning. Jaw tremor was excluded, as it is thought to be distinct from VCMs and not correlated with neuroleptic treatment (Glenthoj et al., 1990). Grooming included forepaw licking, face washing, and body fur grooming as well as scratching. Sniffing included head bobbing and whisker movement. Rearing was characterized as the time spent with both forepaws off the ground and the head elevated. Locomotion was determined by the number of grid crossings.

A separate group of animals was used for each experiment. Each animal was randomly assigned a dose order using a Latin square design and the experimenter was blind to the order of drug treatment. Each animal was tested under all drug conditions separated by a minimum of 48 h. Animals were bilaterally infused with drug or vehicle for 2 min and the infusion cannula was left in place for another 2 min to allow diffusion from the cannula tip. After infusion, animals were replaced in the test boxes and behavioural observation began.
2.4 Post-mortem analysis

Cannula placement was assessed at the end of each experiment. Initially, Sky Blue dye (1.0 µl/side) was infused via the indwelling guide cannulae. The animals were then deeply anaesthetized with sodium pentobarbital (55 mg/kg, i.p.) and perfused with 4% paraformaldehyde. The brains were fixed in this solution for a minimum of 24 h. At the end of this period, the brains were removed and stored at -20°C in a 20% sucrose solution. The brains were then sectioned on a cryostat and the coronal slices (40 µm) stained with Cresyl violet for verification of cannula placement. Only those animals with correct cannula placements were included in the data analysis.

2.5 Data Analysis

Data was analyzed using a two way repeated measures analysis of variance (TREATMENT GROUP x DRUG DOSE, with repeated measures on the antagonist dose). Where significant F-values were found, planned pairwise comparisons were made using a Newman-Keuls test.
3.1 Introduction

The treatment of schizophrenia with chronic antipsychotics leads to the development of tardive dyskinesia (TD), a disorder characterized by involuntary movements of the orobuccal region, in approximately 20-30% of patients (Kane & Smith, 1982; Yassa & Jeste, 1992). A recent study conducted with 200 inpatients found that the prevalence rate of TD was 22% (Modestin et al., 2000). The same study also found that treatment with clozapine, an atypical antipsychotic, was unable to produce a beneficial effect on TD in the patient population (Modestin et al., 2000). Furthermore, while reported cases of TD associated with atypical antipsychotics largely occur in patients with previous histories of long-term typical neuroleptic treatment, a recent case of TD due to treatment with quetiapine, an atypical antipsychotic, in a patient with no previous exposure to typical neuroleptics has been reported (Ghaemi & Ko, 2001). Thus, it is crucial to examine the mechanisms involved in the emergence of TD by chronic neuroleptic treatment in order to provide means by which to counteract the development of this drug-induced disorder.

While the precise mechanisms that contribute to the development of TD remain elusive, it has been suggested that alterations in the balance of activity between the direct striatonigral and the indirect striatopallidal projections of the basal ganglia may lead to the emergence of dyskinesias (Trugman et al., 1996). In order to clarify the specific contribution of these pathways in the development of TD, a rodent model, vacuous chewing movements (VCMs) induced by chronic neuroleptic therapy, has been developed and widely used (Waddington et al., 1983; Stoessl et al., 1989, 1993; Glenthoj et al., 1990; Tamminga et al., 1990; Diana et al., 1992; Egan et al., 1994; Andreassen & Jorgensen, 1995; Andreassen et al., 1996, 1998; Egan et al., 1996;
Meshul et al., 1996; Stoessl, 1996; Chakos et al., 1998; Hashimoto et al., 1998; Bower et al., 2000; Meredith et al., 2000; Van Kampen & Stoessl, 2000).

Among the numerous neurotransmitters endogenous to the nuclei of the basal ganglia, opioid peptides are highly represented (Graybiel, 1990). Furthermore, multiple sub-types of opioid receptors are expressed in the basal ganglia. Dynorphin (DYN), whose protein precursor is preproenkephalin-B (PPE-B), binds selectively to kappa (κ)-opioid receptors and is highly expressed in the direct striatonigral pathway; whereas enkephalin (ENK), whose protein precursor is preproenkephalin-A (PPE-A), binds to both mu (μ)- and delta (δ)-opioid receptors and is expressed in the indirect striatopallidal pathway. These opioid peptides may play a role in the pathogenesis of dyskinesias, given that increased levels of both DYN (Egan et al., 1994, 1996) and ENK mRNA (Hong et al., 1978; Tang et al., 1983; Auchus & Pickel, 1992) as well as morphological changes in enkephalinergic synaptic boutons (Mijnster et al., 1996) have been reported following exposure to chronic neuroleptics.

Increased levels of DYN may be attributed to increased activity in the dopamine D₁ receptor-regulated neurons of the striatonigral pathway. Previous work has indicated that in the dopamine-denervated striatum, intermittent treatment with levodopa or with dopamine D₁ agonists leads to a significant increase in DYN mRNA levels in the striatonigral projection neurons (Gerfen et al., 1990; Steiner & Gerfen, 1996). Furthermore, we have previously shown that intra-nigral infusion of the selective κ-opioid receptor antagonist, nor-binaltorphimine (nor-BNI), attenuates sensitization to rotation caused by intermittent levodopa in 6-OHDA lesioned animals, a putative model of levodopa-induced dyskinesia (Newman et al., 1997). We therefore surmised that the κ-opioid receptor may also play a pivotal role in TD and hypothesized that blockade of nigral κ-opioid receptors might attenuate chronic neuroleptic-induced VCMs.
The opioid peptide ENK is expressed primarily in the indirect striatopallidal pathway, whose neurons are inhibited by stimulation of dopamine D_2 receptors. We have previously shown that subcutaneous injection of the non-specific opioid antagonist naloxone (NAL) attenuates VCMs in animals treated with chronic neuroleptics (Stoessl et al., 1993). However, since peripheral administration may have widespread effects and naloxone acts at all opioid receptor sub-types, the specific central nuclei and opioid receptor subtype involved have yet to be identified. In order to clarify whether attenuation of VCMs by naloxone might also result from blockade of opioid receptors in the indirect pathway as well as to clarify the subtype of opioid receptors which may be implicated in this effect, we examined the effects of selective μ- and δ-opioid receptor antagonists on VCMs following administration directly into the GP.

3.2 Experimental Procedures

3.2.1 Animals and Surgery

The animals used in each experiment weighed approximately 300g at the start of treatment. Thirty-six pairs of male Sprague Dawley rats (Animal Care Centre Breeding Unit, South Campus, UBC) were housed in plexiglas shoebox cages in a temperature-controlled environment with a 12 h light/dark cycle (lights on at 0700 h). The animals had ad libitum access to water but were food restricted (45g/d) to minimize obesity. The animals received a minimum of 7 injections (every 3 weeks for 18 weeks) of fluphenazine decanoate (FLU)(25 mg/kg, i.m.; Squibb, Que) or its vehicle (VEH)(1ml/kg, i.m.; Sigma, St. Louis, MI), sesame oil, before experimental treatment. Animals were treated in accordance with Canadian Council on Animal Care guidelines and all efforts were made to minimize animal suffering and the number of animals.

For studies of the direct striatonigral pathway, animals were pretreated with diazepam (2.5 mg/kg, i.m.; Roche, Ont), anaesthetized with 0.1% halothane gas, and placed in a Kopf
stereotaxic frame. Each animal was implanted with bilateral stainless steel guide cannulae (21 gauge) terminating 2.0 mm above the substantia nigra pars reticulata (SNr) using coordinates derived from Paxinos and Watson (1986) (AP -5.3, ML ± 2.2, DV - 5.7) with reference to Bregma and the skull surface. The cannulae were secured to the skull with jewelers’ screws and acrylic dental cement. Stainless steel obturators were inserted at the end of surgery in order to maintain the patency of the cannulae. Following surgery, a recovery period of a week was allotted before the behavioural testing began.

For studies of the indirect striatopallidal pathway, the animals were treated as above; however, in this case, the guide cannulae (21 gauge) were positioned 2.0 mm above the globus pallidus (GP) (AP -1.3, ML ± 3.2, DV - 5.0).

3.2.2 Materials

Nor-BNI (Sigma, Ont) was dissolved in 0.9% saline. The doses (0, 1.0, 2.0, and 5.0 nmol/µl) selected on the basis of our previous studies [Newman et al., 1997]) were bilaterally infused into the SNr at a rate of 0.5 µl/min. The pH of each dose was checked and was not found to significantly differ from that of physiological saline.

NAL (RBI, MA) (0, 0.5, 2.0 and 5.0 nmol/µl) was dissolved in 0.9% saline and bilaterally infused into the GP at the rate of 0.5 µl/min. The pH of each dose was checked and was not found to significantly differ from that of physiological saline.

D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr Amide (CTOP, the specific µ-opioid antagonist) (RBI, MA) was dissolved in distilled water and bilaterally administered at doses of 0, 0.25, 0.5, and 1.0nmol/µl. The pH of each dose was checked and was not found to significantly differ from that of distilled water.
The specific δ-opioid antagonist naltrindole (NTI) (RBI, MA) was dissolved in 0.9% saline and administered as above (0, 0.25, 0.5, and 1nmol per side). The pH of each dose was checked and was not found to significantly differ from that of physiological saline.

3.2.3 Behavioural testing

A separate group of animals was used for each experiment. The animals were tested in pairs and each animal was tested under all drug conditions separated by a minimum of 48h. Each animal was randomly assigned a dose order using a Latin square design and the experimenter was blind to the order of drug treatment.

On the test day, the animals were placed individually in plexiglas boxes (50 x 50 x 30 cm) for a minimum of 1 h. Following this habituation period, each animal was bilaterally infused using a Harvard Apparatus syringe infusion pump with drug or vehicle for 2 min and the infusion cannula left in place for another 2 min to allow for diffusion from the cannula tip. After infusion, the animals were replaced in the test boxes and observed continuously for three minutes out of every six minute block, alternating between two animals, for a total of 10 blocks (60 min) each. The frequency and duration of various behavioural responses were recorded using a microcomputer keyboard and custom-designed software (BEBOP: Dr. M. Martin-Iverson, University of Western Australia). For each response of interest, a coded key was pressed at the onset and offset of each behaviour. After testing the animals were returned to their home cages and a minimum of 48 h elapsed before re-testing.

The following behavioural responses were recorded by a single rater: VCMs, grooming, locomotion, sniffing, and rearing. VCMs were defined as all undirected mouth movements including chewing and tongue protrusions while excluding jaw tremor and directed movements such as licking, eating, grooming, and yawning. Jaw tremor was excluded, as it is thought to be distinct from VCMs and not correlated with neuroleptic treatment (Glenthoj et al., 1990).
Furthermore, total duration of VCMs was the rating method used. As VCMs tend to occur in bursts, we have found that total duration of VCMs is more likely to reflect the behaviour rather than total frequency of VCMs. Grooming included forepaw licking, face washing, and body fur grooming as well as scratching. Sniffing was characterized by head bobbing and whisker movement. Rearing was defined as the time spent with both forepaws off the ground and the head elevated. The number of grid crossings defined locomotion.

3.2.4 Post-mortem analysis

At the end of the final behavioural observation, Sky Blue dye (1.0 µl/side) was infused via the indwelling guide cannulae using a Harvard Apparatus syringe infusion pump. The animals were then deeply anaesthetized with sodium pentobarbital (55 mg/kg, i.p.; MT. Pharmaceuticals, Ont) and perfused with 4% paraformaldehyde. The brains were fixed in this solution for a minimum of 24 h. At the end of this period, the brains were removed and stored at -20° C in a 20% sucrose solution. The brains were then sectioned on a cryostat and the coronal slices (40 µm) stained with Cresyl violet for verification of cannula placement. Only those animals with correct cannula placements were included in the data analysis (Figs. 2 and 3).

3.2.5 Data analysis

Data were analyzed using a two way repeated measures analysis of variance (FLUPHENAZINE x ANTAGONIST, with repeated measures on the antagonist dose). Where significant F-values were found, planned pairwise comparisons were made using a Newman-Keuls test.

3.3 Results

3.3.1 Effects of the κ-opioid antagonist nor-Binaltorphimine administered intra-nigrally on neuroleptic-induced VCMs

Animals treated with fluphenazine decanoate (25 mg/kg, i.m.) exhibited a significantly increased duration of VCMs compared to animals treated with its vehicle, (N = 15)(F1,13 = 24.12,
Fig. 2. Cannulae placements for intra-nigral infusion of *nor*-binaltorphimine

Fig. 2. Cannulae placements, as indicated by Sky Blue dye infusion, determined from Cresyl Violet-stained coronal sections of the animals used in the *nor*-binaltorphimine experiment through the SNr (5.30, 5.60, and 5.80 mm posterior to bregma). Only those sections from animals with accurately placed cannulae are shown.
Fig. 3. Cannulae placements for intra-pallidal infusion of naloxone, CTOP, or naltrindole

Fig. 3. Cannulae placements, indicated by Sky Blue dye infusion, determined from Cresyl Violet-stained coronal sections of the animals used in the naloxone, CTOP, or naltrindole experiments through the GP (0.92, 1.30, and 1.40 mm posterior to bregma). Only those sections from animals with accurately placed cannulae are shown.
p < 0.001, FLUPHENAZINE main effect) (Fig. 4). Fluphenazine-induced potentiation of VCMs was blocked by intra-nigral infusion of nor-BNI at the 5.0 nmol dose (F_{3,39} = 4.48, p < 0.01, TREATMENT main effect; F_{3,39} = 1.27, p = NS, FLUPHENAZINE X TREATMENT interaction effect) (Fig. 4) but not by nor-BNI at the 1.0 or 2.0 nmol doses. When the data from animals with incorrect cannulae placements were examined, it was found that while there was a significant difference in the duration of VCMs between the vehicle and fluphenazine groups, treatment with nor-BNI had no effect on VCMs.

Locomotion, rearing, sniffing, and grooming were all unaffected by treatment with either fluphenazine or nor-BNI (Table 1).

3.3.2 Effects of intra-pallidal naloxone on neuroleptic-induced VCMs

Animals treated chronically with fluphenazine decanoate (25 mg/kg, i.m.) displayed a significantly higher duration of VCMs than those treated with vehicle (N = 14) (F_{1,12} = 7.14, p < 0.05, FLUPHENAZINE main effect) (Fig. 5). These VCMs were significantly attenuated by treatment with the non-specific opioid antagonist NAL at 0.5 nmol and 2.0 nmol doses but were unaffected by NAL at the 5.0 nmol dose (F_{3,36} = 4.01, p < .05 TREATMENT main effect; F_{3,36} = 3.54, p < 0.05, FLUPHENAZINE x TREATMENT interaction effect) (Fig. 5). When the data from animals with incorrect cannulae placements were examined, it was found that while there was a significant difference between the vehicle and fluphenazine groups in the duration of VCMs, treatment with NAL had no effect on VCMs.

Locomotion, rearing, sniffing, and grooming were all unaffected by treatment with either fluphenazine or NAL (Table 2).

3.3.3 Effects of intra-pallidal infusion of the specific µ-opioid receptor antagonist D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr Amide on neuroleptic induced VCMs
Fig. 4. The effects of intra-nigral infusion of nor-binaltorphimine on VCMs

Fig. 4. The effects of intra-nigral nor-binaltorphimine infusion on VCMs. Chronic treatment with the neuroleptic fluphenazine decanoate induced VCMs. This behaviour was suppressed by intra-nigral infusion of 5.0 nmol/µl nor-binaltorphimine (nor-BNI). Each bar represents the mean (+S.E.M.) duration (s) of VCMs recorded over a 1-h period.
**Significantly different from chronic vehicle treatment, \( p < 0.01 \).
### Significantly different from fluphenazine decanoate + saline treatment, \( p < 0.01 \).
Table 1
The effects of intra-nigral nor-BNI (0.0, 1.0, 2.0, 5.0 nmol/μl) on select behaviours.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Fluphenazine main effect</th>
<th>Treatment main effect</th>
<th>Interaction Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locomotion</td>
<td>$F_{1,13} = 0.51, p = \text{NS}$</td>
<td>$F_{3,39} = 1.26, p = \text{NS}$</td>
<td>$F_{3,39} = 0.70, p = \text{NS}$</td>
</tr>
<tr>
<td>Rearing</td>
<td>$F_{1,13} = 0.64, p = \text{NS}$</td>
<td>$F_{3,39} = 0.07, p = \text{NS}$</td>
<td>$F_{3,39} = 2.09, p = \text{NS}$</td>
</tr>
<tr>
<td>Sniffing</td>
<td>$F_{1,13} = 0.27, p = \text{NS}$</td>
<td>$F_{3,39} = 0.64, p = \text{NS}$</td>
<td>$F_{3,39} = 1.36, p = \text{NS}$</td>
</tr>
<tr>
<td>Grooming</td>
<td>$F_{1,13} = 0.87, p = \text{NS}$</td>
<td>$F_{3,39} = 1.17, p = \text{NS}$</td>
<td>$F_{3,39} = 0.25, p = \text{NS}$</td>
</tr>
</tbody>
</table>

$N = 7, \text{Vehicle;} N = 8, \text{Fluphenazine.}$
Fig. 5. The effects of intra-pallidal naloxone infusion on VCMs

Fig. 5. The effects of intra-pallidal naloxone infusion on VCMs. Chronic treatment with the neuroleptic fluphenazine decanoate resulted in VCMs. This behaviour was attenuated by intra-pallidal infusion of naloxone (NAL) at 0.5 and 2.0 nmol/µl but was unaffected at the 5.0 nmol/µl dose. Each bar represents the mean (+ S.E.M.) duration (s) of VCMs recorded over a 1-h period. ** Significantly different from chronic vehicle treatment, p < 0.01. ## Significantly different from fluphenazine decanoate + saline treatment, p < 0.01.
Table 2
The effects of intra-pallidal NAL (0.0, 0.5, 2.0, 5.0 nmol/μl) on select behaviours.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Fluphenazine main effect</th>
<th>Treatment main effect</th>
<th>Interaction Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locomotion</td>
<td>$F_{1,12} = 2.22, p = \text{NS}$</td>
<td>$F_{3,36} = 0.49, p = \text{NS}$</td>
<td>$F_{3,36} = 0.43, p = \text{NS}$</td>
</tr>
<tr>
<td>Rearing</td>
<td>$F_{1,12} = 0.55, p = \text{NS}$</td>
<td>$F_{3,36} = 1.55, p = \text{NS}$</td>
<td>$F_{3,36} = 0.26, p = \text{NS}$</td>
</tr>
<tr>
<td>Sniffing</td>
<td>$F_{1,12} = 0.36, p = \text{NS}$</td>
<td>$F_{3,36} = 0.21, p = \text{NS}$</td>
<td>$F_{3,36} = 0.05, p = \text{NS}$</td>
</tr>
<tr>
<td>Grooming</td>
<td>$F_{1,12} = 0.06, p = \text{NS}$</td>
<td>$F_{3,36} = 0.99, p = \text{NS}$</td>
<td>$F_{3,36} = 0.73, p = \text{NS}$</td>
</tr>
</tbody>
</table>

N = 7, Vehicle; N = 7, Fluphenazine.
The animals receiving chronic fluphenazine showed increased duration of VCMs compared to animals receiving vehicle (N = 11)(F_{1,9} = 25.34, p < 0.001, FLUPHENAZINE main effect)(Fig. 6). This effect was blocked by treatment with CTOP at 0.5 and 1.0 nmol but not by 0.25 nmol (F_{3,27} = 8.99, p < 0.001, TREATMENT main effect; F_{3,27} = 10.94, p < 0.001, FLUPHENAZINE x TREATMENT interaction effect)(Fig. 6). When the data from animals with incorrect cannulae placements were examined, it was found that while there was a significant difference in the duration of VCMs between the vehicle and fluphenazine groups, treatment with CTOP had no effect on VCMs.

Of the other behaviours examined only locomotion was affected by chronic fluphenazine treatment. Rearing, sniffing, and grooming were all unaltered by treatment with either fluphenazine or CTOP (Table 3).

3.3.4 Effects of the specific δ-opioid receptor antagonist naltrindole, administered into the globus pallidus, on neuroleptic induced vacuous chewing movements

Animals receiving fluphenazine developed VCMs of increased duration compared to those animals treated with vehicle (N = 14)(F_{1,12} = 47.43, p < 0.001, FLUPHENAZINE main effect)(Fig. 7). This effect was effectively attenuated by all three doses of NTI (0.25, 0.50, and 1.0 nmol/µl)(F_{3,36} = 6.03, p < 0.01, TREATMENT main effect; F_{3,36} = 5.62, p < 0.01, FLUPHENAZINE x TREATMENT interaction effect)(Fig. 7). When the data from animals with incorrect cannulae placements were examined, it was found that while there was a significant difference between the vehicle and fluphenazine groups in the duration of VCMs, treatment with NTI had no effect on VCMs.

Locomotion was significantly attenuated by chronic treatment with fluphenazine (F_{1,12} = 17.63, p < 0.01, FLUPHENAZINE main effect), an effect which was also seen to a lesser extent following treatment with NTI (F_{3,36} = 3.12, p < 0.05, TREATMENT main effect)(Table 4).
Fig. 6. The effects of intra-pallidal infusion of CTOP on VCMs

Fig. 6. The effects of intra-pallidal infusion of CTOP on VCMs. Chronic fluphenazine decanoate treatment resulted in the development of VCMs. Infusion of 0.5 and 1.0 nmol/μl of the selective μ-opioid antagonist CTOP into the GP resulted in the attenuation of VCMs; however, the 0.25 nmol/μl dose of CTOP was ineffective. Each bar represents the mean (+S.E.M.) duration (s) of VCMs recorded over a 1-h period.

** Significantly different from chronic vehicle treatment, $p < 0.01$.

## Significantly different from fluphenazine decanoate + saline treatment, $p < 0.01$. 
Table 3
The effects of intra-pallidal CTOP (0.0, 0.25, 0.5, 1.0 nmol/µl) on select behaviours.

<table>
<thead>
<tr>
<th></th>
<th>Fluphenazine main effect</th>
<th>Treatment main effect</th>
<th>Interaction Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locomotion</td>
<td>$F_{1.9} = 5.30, p &lt; 0.05$</td>
<td>$F_{3.27} = 0.37, p = NS$</td>
<td>$F_{3.27} = 0.54, p = NS$</td>
</tr>
<tr>
<td>Rearing</td>
<td>$F_{1.9} = 2.02, p = NS$</td>
<td>$F_{3.27} = 1.19, p = NS$</td>
<td>$F_{3.27} = 0.72, p = NS$</td>
</tr>
<tr>
<td>Sniffing</td>
<td>$F_{1.9} = 0.67, p = NS$</td>
<td>$F_{3.27} = 0.50, p = NS$</td>
<td>$F_{3.27} = 0.31, p = NS$</td>
</tr>
<tr>
<td>Grooming</td>
<td>$F_{1.9} = 0.69, p = NS$</td>
<td>$F_{3.27} = 0.78, p = NS$</td>
<td>$F_{3.27} = 0.11, p = NS$</td>
</tr>
</tbody>
</table>

$N = 6$, Vehicle; $N = 5$, Fluphenazine.
Fig. 7. The effects of intra-pallidal infusion of naltrindole on VCMs

Fig. 7. The effects of intra-pallidal infusion of naltrindole on VCMs. Chronic treatment with fluphenazine decanoate resulted in VCMs. These movements were suppressed by intra-pallidal infusion of the selective δ-opioid receptor antagonist naltrindole (NTI) at all three doses (0.25, 0.50, and 1.0 nmol/μl). Each bar represents the mean (+S.E.M.) duration (s) of VCMs recorded over a 1-h period.

** Significantly different from chronic vehicle treatment, \( p < 0.01 \).

### Significantly different from fluphenazine decanoate + saline treatment, \( p < 0.01 \).
## Table 4

The effects of intra-pallidal NTI treatment on total time (s) engaged in locomotion.

<table>
<thead>
<tr>
<th>Group</th>
<th>0.0 nmol/μl</th>
<th>0.25 nmol/μl</th>
<th>0.50 nmol/μl</th>
<th>1.0 nmol/μl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>12.17 ± 3.30</td>
<td>2.67 ± 1.41</td>
<td>6.00 ± 3.30</td>
<td>4.67 ± 2.55</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>1.50 ± 0.89</td>
<td>1.50 ± 0.89</td>
<td>1.00 ± 0.57</td>
<td>0.13 ± 0.13</td>
</tr>
</tbody>
</table>

Values represent the mean (± S.E.M.) duration of responses (s) scored over 10 blocks of 3 min. $F_{1,12} = 17.63, p < 0.01$ (Fluphenazine main effect). $F_{3,36} = 3.12, p < 0.05$ (Treatment main effect). $F_{3,36} = 2.56, p = NS$ (Interaction effect). $N = 6$, Vehicle; $N = 8$, Fluphenazine.
addition, rearing, sniffing, and grooming were all reduced by chronic fluphenazine treatment in this group of animals but were unaffected by NTI treatment (Table 5).

3.4 Discussion

As expected, chronic treatment with neuroleptics resulted in a robust development of VCMs in animals receiving FLU compared to those treated with VEH in all four experiments. While animals were used repeatedly within each experiment, different groups of animals were used for each and this may account for the disparity in response rates across experiments. As well, while there was a disparity in guide cannula placement within the GP, this was not found to have a consistent effect on behaviour either within or across experiments. A suppression of locomotion, a behaviour that may be inversely correlated with the development of VCMs (Levy et al., 1987), was also seen in the chronic FLU animals in a number of the studies. However, the failure of this effect to appear consistently across all experiments as well as the failure of opioid antagonists to consistently affect locomotor counts suggests that the effect of opioid antagonists on VCMs was a true effect, rather than the result of behavioural response competition.

Given the small volume and slow rate of infusion, it is likely that the diffusion of drug was approximately one millimeter from the infusion site. It is, therefore, unlikely that the results can be attributed to drug effects on other brain structures than those targeted. Furthermore, when data from animals with incorrect cannulae placements were examined, it was found that while there was a significant difference in the duration of VCMs between the fluphenazine and vehicle groups, drug treatment had no effect on VCMs. Thus, drug infusion into other brain structures had no effect on VCMs.

The compounds used have high affinity for the opioid receptors examined in each of the current studies. For example, nor-BNI binds selectively to the κ-opioid receptor and has a low affinity μ- and δ-opioid receptor (Portoghese et al., 1987). In fact, nor-BNI has 100 times greater
Table 5
The effects of intra-pallidal NTI (0.0, 0.25, 0.5, 1.0 nmol/μl) on select behaviours.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Fluphenazine main effect</th>
<th>Treatment main effect</th>
<th>Interaction Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rearing</td>
<td>$F_{1,12} = 38.28, p &lt; 0.001$</td>
<td>$F_{3,36} = 1.49, p = NS$</td>
<td>$F_{3,36} = 1.19, p = NS$</td>
</tr>
<tr>
<td>Sniffing</td>
<td>$F_{1,12} = 12.05, p &lt; 0.01$</td>
<td>$F_{3,36} = 1.29, p = NS$</td>
<td>$F_{3,36} = 0.29, p = NS$</td>
</tr>
<tr>
<td>Grooming</td>
<td>$F_{1,12} = 17.56, p &lt; 0.01$</td>
<td>$F_{3,36} = 0.98, p = NS$</td>
<td>$F_{3,36} = 0.56, p = NS$</td>
</tr>
</tbody>
</table>

N = 6, Vehicle; N = 8, Fluphenazine.
κ/μ selectivity ratio that naltrexone (Portoghese et al., 1987). CTOP is also highly selective for its receptor, the μ-opioid receptor. It is approximately 4000-fold more selective for the μ- versus δ-opioid receptor in rat brain (Pelton et al., 1986). The antagonist NTI is selective for the δ-opioid receptor; it is 66-fold more selective for δ- vs. μ-opioid receptors and 250-fold more selective for δ- vs. κ-opioid receptors (Rogers et al., 1989). While it may have been interesting to examine the effects of nor-BNI in the GP and both CTOP and NTI in the SNr, the distribution of κ-, δ-, and μ-opioid receptors differs quite widely in the basal ganglia (Sharif & Hughes, 1989).

Treatment with opioid receptor antagonists displayed varying potency in suppressing VCMs resulting from chronic neuroleptic therapy, depending upon the compound used. The κ-opioid receptor antagonist nor-BNI blocked VCMs at only the highest dose (5.0 nmol/μl) when administered into the SNr. NAL, a non-specific opioid receptor antagonist, successfully attenuated VCMs when infused into the GP at doses of 0.5 nmol and 2.0 nmol/μl but had no effect at a higher dose (5.0 nmol/μl). The μ-opioid receptor antagonist CTOP inhibited VCMs when infused into the GP at 0.5 nmol and 1.0 nmol/μl but not at 0.25 nmol/μl. Finally, intrapallidal infusion of NTI, the selective δ-opioid receptor antagonist, attenuated VCMs at all doses studied.

The ability of nor-BNI to attenuate VCMs at the 5.0 nmol/μl dose suggests that increased DYN in the striatonigral pathway following chronic administration of neuroleptics is a likely contributor to the emergence of TD. This finding concurs with those in a rodent model of Parkinson’s disease, where increased DYN appears to play a crucial role in the expression of behavioural sensitization to intermittent levodopa treatment (Newman et al., 1997). This result is also in keeping with increased expression of DYN mRNA previously associated with chronic neuroleptic therapy (Egan et al., 1994).
Steiner and Gerfen (1998) have suggested that DYN may act as a negative feedback mechanism to prevent the excessive stimulation of striatonigral outflow neurons by dopamine. While this may be true at the level of the striatum, increased stimulation of nigral \( \kappa \)-opioid receptors may also inhibit glutamate release from subthalamoniagral projection neurons, thereby resulting in disinhibition of motor nuclei of the thalamus (Maneuf et al., 1995). A consequence of this disinhibition could be the development of TD.

Striatopallidal ENK also appears to play a critical role in the emergence of TD, as opioid antagonists infused into the GP were able to suppress VCMs. While it has previously been demonstrated that systemic administration of NAL suppresses neuroleptic-induced VCMs (Stoessl et al., 1993) as well as neuroleptic-induced oral stereotypies (Pollock & Kornetsky, 1991), the localization of this effect is crucial to our understanding of the pathogenesis of TD. The inability of NAL to attenuate VCMs at the highest dose administered (5.0 nmol/\( \mu \)l) may reflect actions of this antagonist at sites other than opioid receptors. As the dose was large, diffusion of NAL may have permitted sufficient quantified of the drug to interact with receptors other than those targeted.

We attempted to determine the specific pallidal opioid receptor subtypes mediating the emergence of VCMs after chronic neuroleptic treatment by using the selective \( \mu \)- and \( \delta \)-antagonists, CTOP and NTI respectively. Our results suggest that both \( \mu \)- and \( \delta \)-opioid receptors play a pivotal role, as both compounds effectively blocked the expression of VCMs.

The increase in ENK mRNA levels following chronic treatment with neuroleptics suggests increased activation of the striatopallidal pathway (Egan et al., 1994); thus compensatory changes in receptor density of the globus pallidus may be expected. However, recent evidence has suggested that while a decrease in \( \mu \)-opioid receptor binding is found in rats treated with chronic haloperidol, this reduction in opioid receptors was unconnected to the
development of VCMs (Bower et al., 2000). Nevertheless, the effect of opioid receptor blockade in the studies reported here may reflect an action on striatopallidal terminals. Opioid receptor stimulation results in reduced GABA release in the GP (Maneuf et al., 1994). This could serve to inhibit the subthalamic nucleus, internal globus pallidus (entopeduncular nucleus in the rat) and SNr, resulting in disinhibition of thalamic motor nuclei, an effect prevented by pallidal opioid receptor blockade.

While increases in both DYN and ENK appear to play crucial roles in the emergence of TD, the effect of κ-opioid receptor blockade was incomplete. Only the highest dose of nor-BNI (5.0 nmol) significantly suppressed VCMs. This may suggest that while increased DYN could contribute to the emergence of TD, it may not be sufficient to produce it. However, whatever the relative contribution of DYN or ENK to the emergence of TD, the finding that opioid antagonists infused into either the direct striatonigral pathway or the indirect striatopallidal pathway will attenuate neuroleptic-induced VCMs corroborates the suggestion that alterations in the activity of either of these pathways may lead to the development of dyskinesias (Trugman et al., 1996).

The rodent model of TD used in these experiments, vacuous chewing movements (VCMs) induced by chronic neuroleptic therapy, remains controversial. Some researchers have argued that VCMs are more analogous to Parkinsonian tremor (Jicha & Salamone, 1991) or acute dystonia (Rupniak et al., 1985) than to TD. This controversy notwithstanding, there are similarities between chronic neuroleptic-induced VCMs and TD which support its use as a putative model of TD: VCMs develop along similar time lines to TD (Glenthoj et al., 1990); VCMs persist following withdrawal from medication (Waddington et al., 1983; Ellison, 1991); and they are suppressed by treatment with dopamine receptor antagonists (Stoessl et al., 1989) and by dopamine depletion (Diana et al., 1992).
The development of VCMs along similar time lines to TD is an important issue in the evaluation of this rodent model. While some studies have found the emergence of VCMs early on in the course of treatment, other studies have revealed these movements to be qualitatively different from the VCMs which emerge following long term neuroleptic treatment (Andreassen & Jorgensen, 1995; Egan et al., 1996). Thus, in our studies, the animals were not observed for VCMs during the initial fluphenazine treatment period.

The quantification of VCMs is scored in different ways by groups using this rodent model of TD. While a number of groups use frequency of VCMs as a measure, our lab scores the total duration of VCMs over the testing period. This measure has been widely used in our lab (Stoessl et al., 1989, 1993; Stoessl, 1996; Van Kampen & Stoessl, 2000) and was carried out by a single rater. Given the tendency of VCMs to occur in bursts, we have found that total duration of VCMs is more likely to be reflective of the behaviour than total frequency.

The results of these experiments have relevance to clinical therapeutic regimes because while newer antipsychotic treatments, which are less likely to result in the development of TD, do exist, older generation antipsychotics are still widely used in the treatment of schizophrenia. These findings suggest that opioid receptor antagonists may play a role in the treatment of extrapyramidal side effects resulting from chronic neuroleptic treatment.
CHAPTER IV Blockade of neurotensin receptors in tardive dyskinesia

4.1 Introduction

The treatment of schizophrenia with chronic neuroleptics leads to the development of tardive dyskinesia (TD), a disorder characterized by involuntary movements of the orobuccal region, in approximately 20-30% of patients (Kane & Smith, 1982; Yassa & Jeste, 1992). A recent study conducted in 200 in-patients confirmed this figure (Modestin et al., 2000). Importantly, this study reported that treatment with the atypical antipsychotic clozapine was without benefit for TD (Modestin et al., 2000). Moreover, while reported cases of TD associated with atypical antipsychotics have usually been seen in patients previously treated with typical antipsychotics, this is not always the case. A recent case of TD resulting from treatment with the atypical neuroleptic quetiapine in a patient with no previous exposure to typical neuroleptics has been reported (Ghaemi & Ko, 2001). Thus, it remains crucial to examine the mechanisms involved in the emergence of TD following chronic neuroleptic treatment.

While the precise mechanisms that contribute to the development of TD remain elusive, it has been suggested that alterations in the balance of activity between the direct striatonigral and the indirect striatopallidal projections of the basal ganglia may lead to the emergence of dyskinesias (Trugman et al., 1996). A rodent model of vacuous chewing movements (VCMs) induced by chronic neuroleptic therapy has been widely used and may help to clarify the mechanisms involved in the development of TD (Iversen et al., 1980; Waddington et al., 1983; Stoessl et al., 1989, 1993; Glenthoj et al., 1990; Tamminga et al., 1990; Diana et al., 1992; Andreassen & Jorgensen, 1995; Stoessl, 1995; Egan et al., 1996; Chakos et al., 1998; Hashimoto et al., 1998; Van Kampen & Stoessl, 2000; McCormick & Stoessl, 2002).

There are numerous neurotransmitters endogenous to the output pathways of the basal ganglia. Peptides, both opioid and non-opioid, are among them (Graybiel, 1990). Neurotensin
(NT) is a tridecapeptide which is expressed in both dopamine D1-receptor bearing neurons of the direct striatonigral pathway and dopamine D2-receptor bearing neurons of the indirect striatopallidal pathway (Castel et al., 1993, 1994; Hökfelt et al., 2000).

Changes in NT levels resulting from long-term antipsychotic therapy suggest a possible role for NT in the emergence of TD. In particular, changes in the expression of NT and its receptors following chronic neuroleptic treatment differ depending on whether typical or atypical antipsychotics are administered. For instance, increased release and tissue concentration of NT occur in the nucleus accumbens (NAcc) only following long-term therapy with atypical antipsychotics, whereas increases occur in both the NAcc and the caudate nucleus following typical antipsychotics (Goedert et al., 1985; Radke et al., 1989, 1998). As well, differences in NT receptor binding in the NAcc are found only with atypical antipsychotics, while typical antipsychotics alter NT receptor binding in the striatum alone (Holtom et al, 2000). In addition, NT receptor binding is increased in the substantia nigra and NT receptor mRNA levels increase in both the substantia nigra and the ventral tegmental area following chronic treatment with typical antipsychotics (Uhl & Kuhar, 1984; Bolden-Watson et al., 1993), while they remain unchanged following treatment with atypical antipsychotics (Bolden-Watson et al., 1993). Thus, it is possible that the alterations found in association with chronic atypical antipsychotic therapy may be related to the clinical efficacy of treatment (Binder et al., 2001), as these drugs have a lower incidence of extrapyramidal side effects. In contrast, those additional changes seen following typical antipsychotics may play a role in the emergence of extrapyramidal side effects such as TD (Radke et al., 1998; Kinkead et al, 1999).

Dopamine depletion with reserpine and dopamine D2 receptor blockade with haloperidol both result in increased expression of NT mRNA in the direct striatonigral and the indirect striatopallidal output pathways of the basal ganglia (Brog & Zahm, 1996). As well,
intracerebroventricular (ICV) NT potentiates VCMs in animals treated with chronic neuroleptics (Stoessl & Szczutkowski, 1991) and NT administered directly into the ventrolateral striatum elicits VCMs in neuroleptic-naïve animals (Stoessl, 1995). This evidence, together with the fact that the ventrolateral striatum has been previously associated with the emergence of oral dyskinesias (Mackay, 1982; Pisa, 1988; Kelley et al., 1989; Salamone et al., 1990), suggests the importance of NT in this region for the expression of neuroleptic-induced VCMs. Furthermore, as chronic neuroleptic therapy can result in increased NT mRNA in both the direct striatonigral and the indirect striatopallidal output pathways, we attempted to determine which projection is more important for the induction of dyskinesias, by examining the effects of a NT antagonist infused into their target regions.

4.2 Experimental Procedures

4.2.1. Animals and Surgery

The animals used in each experiment weighed between 250 - 300g at the start of treatment. Pairs of male Sprague Dawley rats (Animal Care Centre Breeding Unit, South Campus, UBC) were housed in plexiglas shoebox cages in a temperature-controlled environment with a 12 h light/dark cycle (lights on at 0700 h). The animals had ad libitum access to water but were food restricted (45g/d) to minimize obesity. All animals received a minimum of 7 injections (every 3 weeks for 18 weeks) of fluphenazine decanoate (FLU)(25 mg/kg, i.m.; Squibb, Que) or its vehicle (VEH)(1ml/kg, i.m.; Sigma, St. Louis, MI), sesame oil, before experimental treatment. Animals were treated in accordance with Canadian Council on Animal Care guidelines and all efforts were made to minimize animal suffering and the number of animals used.

Animals were pretreated with diazepam (2.5 mg/kg, i.m.; Roche, Ont), anaesthetized with 0.1% halothane gas, and placed in a Kopf stereotaxic frame. Each animal was implanted with
bilateral stainless steel guide cannulae (21 gauge) terminating 2.0 mm above the striatum (AP -0.8, ML ± 4.2, DV - 6.0), the SNr (AP -5.3, ML ± 2.2, DV - 5.7), or the GP (AP -1.3, ML ± 3.2, DV - 5.0). All coordinates were derived from Paxinos and Watson (1986) with reference to Bregma and the skull surface. The cannulae were secured to the skull with jewelers’ screws and acrylic dental cement. Stainless steel obturators were inserted at the end of surgery in order to maintain the patency of the cannulae. Following surgery, a recovery period of a week was allotted before the behavioural testing began.

4.2.2 Materials

The non-peptide NT receptor 1 (NTS1) antagonist 2-[(1-(7-chloro-4-quinolinyl)-5-(2,6-dimethoxyphenyl)pyrazol-3-yl)carbonylamino] tricyclo(3.3.1.1.3.7) decan-2-carboxylic acid (SR48692, a gift from Dr. D. Gully, Sanofi, France) was dissolved in a solution of dimethyl sulfoxide (DMSO) (10%) and 0.9% saline (90%) and sonicated using a Sonicator cell disruptor Model 10 (Heat Systems/Ultrasonics Inc., New York) until the drug was completely suspended in solution. The doses of SR48692 (0.25, 0.50, and 1.0 nmol/μl, selected on the basis of pilot studies) or its vehicle (10% DMSO in saline) were bilaterally infused into the striatum, the SNr, or the GP at a rate of 0.5 μl/min.

4.2.3 Behavioural Testing

A separate group of animals was used for each experiment. The animals were tested in pairs and each animal was tested under all 4 drug conditions, separated by a minimum of 48h. Each animal was randomly assigned a dose order using a Latin square design and the experimenter was blind to the order of drug treatment.

On the test day, the animals were placed individually in plexiglas boxes (50 x 50 x 30 cm) for a minimum of 1 h prior to the start of behavioural observation. Following this habituation period, each animal was bilaterally infused with SR48692 or vehicle for 2 min using
a Harvard Apparatus syringe infusion pump and the infusion cannula left in place for another 2 min to allow diffusion from the cannula tip. After infusion, the animals were replaced in the test boxes and observed continuously for three minutes out of every six minute block, alternating between two animals, for a total of 10 blocks (60 min) each. The frequency and duration of various behavioural responses were recorded using a microcomputer keyboard and custom-designed software (BEBOP; Dr. M. Martin-Iverson, University of Western Australia). For each response of interest, a coded key was pressed at the onset and offset of behaviour. After testing, the animals were returned to their home cages and a minimum of 48 h elapsed before re-testing.

The following behavioural responses were recorded by a single rater: VCMs, grooming, locomotion, sniffing, and rearing. VCMs were defined as all undirected mouth movements including chewing and tongue protrusions while excluding jaw tremor and directed movements such as licking, eating, grooming, and yawning. Jaw tremor was excluded, as it is thought to be distinct from VCMs and not correlated with neuroleptic treatment (Glenthoj et al., 1990). We scored total duration of VCMs because the response tends to occur in bursts, and we have found that duration reflects the behaviour better than frequency measures. Grooming included forepaw licking, face washing, and body fur grooming as well as scratching, but excluded penile grooming. Sniffing was characterized by head bobbing and whisker movement. Rearing was defined as the time spent with both forepaws off the ground and the head elevated. Locomotion was determined by the number of grid crossings.

4.2.4 Post-mortem analysis

At the end of the final behavioural observation, Sky Blue dye (1.0 μl/side) was infused via the indwelling guide cannulae using a Harvard Apparatus syringe infusion pump. The animals were then deeply anaesthetized with sodium pentobarbital (55 mg/kg, i.p.; MT. Pharmaceuticals, Ont) and perfused with 4% paraformaldehyde. The brains were fixed in this
solution for a minimum of 24 h. At the end of this period, the brains were removed and stored at -20°C in a 20% sucrose solution. The brains were then sectioned on a cryostat and coronal slices (40 μm) stained with Creysl violet for verification of cannula placement. Only those animals with correct cannula placements were included in the data analysis (Figs. 8, 9, and 10).

4.2.5 Data analysis

Data were analyzed using a two way repeated measures analysis of variance (FLUPHENAZINE x ANTAGONIST, with repeated measures on the antagonist dose). Where significant F-values were found, planned pairwise comparisons were made using a Newman-Keuls test.

4.3 Results

4.3.1 Effects of the neurotensin receptor antagonist SR48692 administered into the striatum on neuroleptic-induced VCMs

Animals treated with fluphenazine decanoate (25 mg/kg, i.m.) exhibited a significantly greater duration of VCMs compared to animals treated with its vehicle, (N = 16)(F1,14 = 66.42, p < 0.001, FLUPHENAZINE main effect) (Fig. 11). Fluphenazine-induced potentiation of VCMs was attenuated by intra-striatal infusion of all three doses of SR48692 (0.25, 0.50, and 1.0 nmol/μl)(F3,42 = 8.38, p < 0.001, TREATMENT main effect; F3,42 = 7.24, p < 0.001, FLUPHENAZINE x TREATMENT interaction effect)(Fig. 11). In animals with incorrect cannula placements, there was a significant difference in the duration of VCMs between the vehicle and fluphenazine groups, treatment with SR48692 had no effect on VCMs in these animals (data not shown).

Locomotion, rearing, and sniffing were all unaffected by treatment with either fluphenazine or SR48692 (Table 6). However, grooming was reduced by chronic fluphenazine treatment in this group of animals but unaffected by treatment with SR48692 (Table 7).
Fig. 8. Cannulae placements for intra-striatal infusion of SR48692

Fig. 8. Cannulae placements, indicated by infusion of Sky Blue dye, determined from Cresyl Violet-stained coronal sections of the animals used in the SR48692 experiments through the striatum (0.80, 0.92, and 1.30 mm posterior to bregma). Only those sections from animals with accurately placed cannulae are shown.
Fig. 9. Cannulae placements for intra-nigral infusion of
SR48692

Fig. 9. Cannulae placements, indicated by infusion of Sky Blue dye, determined from Cresyl
Violet-stained coronal sections of the animals used in the SR48692 experiments through the SNr
(5.30, 5.60, and 5.90 mm posterior to bregma). Only those sections from animals with correctly
placed cannulae are shown.
Fig. 10. Cannulae placements for intra-pallidal infusion of SR48692

Fig. 10. Cannulae placements, as indicated by Sky Blue dye infusion, determined from Cresyl Violet-stained coronal sections of the animals used in the SR48692 experiments through the GP (0.92, 1.30, and 1.40 mm posterior to bregma). Only those sections from animals with correctly placed cannulae are shown.
Fig. 11. The effects of intra-striatal infusion of SR48692 on VCMs

Chronic treatment with fluphenazine decanoate resulted in the development of VCMs. This behaviour was attenuated by intra-striatal infusion of SR48692 at all three doses (0.25, 0.50, and 1.0 nmol/ml). Each bar represents the mean (+S.E.M.) duration (s) of VCMs recorded over a 1-h period.

** Significantly different from chronic vehicle treatment, \( p < 0.01 \).

* Significantly different from vehicle + 0.25 nmol SR48692, \( p < 0.01 \).

** Significantly different from vehicle + 0.50 nmol SR48692, \( p < 0.01 \).

** Significantly different from vehicle + 1.0 nmol SR48692, \( p < 0.01 \).

### Significantly different from fluphenazine decanoate + 10% DMSO in saline, \( p < 0.01 \).
Table 6
The effects of central infusion of SR48692 (0.0, 0.25, 0.5, 1.0 nmol/μl) on select behaviours.

<table>
<thead>
<tr>
<th>Intra-striatal</th>
<th>Fluphenazine main effect</th>
<th>Treatment main effect</th>
<th>Interaction Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locomotion</td>
<td>$F_{1,14} = 0.36, \ p = \text{NS}$</td>
<td>$F_{3,42} = 0.80, \ p = \text{NS}$</td>
<td>$F_{3,42} = 0.68, \ p = \text{NS}$</td>
</tr>
<tr>
<td>Rearing</td>
<td>$F_{1,14} = 1.71, \ p = \text{NS}$</td>
<td>$F_{3,42} = 1.06, \ p = \text{NS}$</td>
<td>$F_{3,42} = 1.92, \ p = \text{NS}$</td>
</tr>
<tr>
<td>Sniffing</td>
<td>$F_{1,14} = 1.73, \ p = \text{NS}$</td>
<td>$F_{3,42} = 1.26, \ p = \text{NS}$</td>
<td>$F_{3,42} = 0.73, \ p = \text{NS}$</td>
</tr>
<tr>
<td>Intra-nigral</td>
<td>$F_{1,11} = 0.09, \ p = \text{NS}$</td>
<td>$F_{3,33} = 1.03, \ p = \text{NS}$</td>
<td>$F_{3,33} = 2.38, \ p = \text{NS}$</td>
</tr>
<tr>
<td>Locomotion</td>
<td>$F_{1,11} = 0.17, \ p = \text{NS}$</td>
<td>$F_{3,33} = 0.49, \ p = \text{NS}$</td>
<td>$F_{3,33} = 1.61, \ p = \text{NS}$</td>
</tr>
<tr>
<td>Rearing</td>
<td>$F_{1,11} = 1.65, \ p = \text{NS}$</td>
<td>$F_{3,33} = 0.28, \ p = \text{NS}$</td>
<td>$F_{3,33} = 2.16, \ p = \text{NS}$</td>
</tr>
<tr>
<td>Sniffing</td>
<td>$F_{1,11} = 2.19, \ p = \text{NS}$</td>
<td>$F_{3,33} = 1.04, \ p = \text{NS}$</td>
<td>$F_{3,33} = 1.16, \ p = \text{NS}$</td>
</tr>
<tr>
<td>Intra-pallidal</td>
<td>$F_{1,11} = 3.57, \ p = \text{NS}$</td>
<td>$F_{3,33} = 2.24, \ p = \text{NS}$</td>
<td>$F_{3,33} = 1.01, \ p = \text{NS}$</td>
</tr>
<tr>
<td>Locomotion</td>
<td>$F_{1,11} = 0.83, \ p = \text{NS}$</td>
<td>$F_{3,33} = 1.99, \ p = \text{NS}$</td>
<td>$F_{3,33} = 0.30, \ p = \text{NS}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SR48692 (nmol/µl)</th>
<th>0.0</th>
<th>0.25</th>
<th>0.50</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>117.79 ± 26.05</td>
<td>116.73 ± 13.71</td>
<td>136.24 ± 29.72</td>
<td>136.99 ± 37.14</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>61.16 ± 18.33</td>
<td>49.65 ± 16.57</td>
<td>86.83 ± 21.64</td>
<td>82.74 ± 21.24</td>
</tr>
</tbody>
</table>

Values represent the mean (± S.E.M.) duration of responses (s) scored over 10 blocks of 3 min. $F_{1,14} = 6.02, p < 0.05$ (Fluphenazine main effect). $F_{3,42} = 0.98, p = NS$ (Treatment main effect). $F_{3,42} = 0.67, p = NS$ (Interaction effect). N = 8, Vehicle; N = 8, Fluphenazine.
4.3.2 Effects of intra-nigral administration of the neurotensin receptor antagonist SR48692 on neuroleptic-induced VCMs

Animals treated with fluphenazine decanoate (25 mg/kg, i.m.; N = 7) exhibited a significant increase in duration of VCMs compared to animals treated with its vehicle, (N = 6)\(F_{1,11} = 16.91, \ p < 0.01, \) FLUPHENAZINE main effect\) (Fig. 12). Fluphenazine-induced potentiation of VCMs was attenuated by intra-nigral infusion of SR48692 at all three doses (0.25, 0.50, and 1.0 nmol/μl) (\(F_{3,33} = 2.89, \ p < 0.05, \) TREATMENT main effect; \(F_{2,33} = 2.93, \ p < 0.05, \) FLUPHENAZINE X TREATMENT interaction effect)\(\) (Fig. 12). In animals with incorrect cannula placements, there was a significant difference in the duration of VCMs between the vehicle and fluphenazine groups but treatment with SR48692 had no effect (data not shown).

Locomotion, rearing, sniffing, and grooming were all unaffected by treatment with either fluphenazine or SR48692 (Table 6).

4.3.3 Effects of the neurotensin receptor antagonist SR48692 administered into the globus pallidus on neuroleptic-induced vacuous chewing movements

Animals receiving fluphenazine decanoate (N = 7) exhibited VCMs of increased duration compared to those animals treated with vehicle (N = 6)\(F_{1,11} = 18.10, \ p < 0.01, \) FLUPHENAZINE main effect\) (Fig. 13). This effect was effectively attenuated by only the highest dose of SR48692 administered (1.0 nmol/μl)\(F_{3,33} = 3.16, \ p < 0.05, \) TREATMENT main effect; \(F_{3,33} = 2.20, \ p = \text{NS, FLUPHENAZINE x TREATMENT interaction effect, but was unaffected by lower doses of SR48692 (Fig. 13). In animals with incorrect cannula placements, there was a significant difference between the vehicle and fluphenazine groups in the duration of VCMs, but treatment with SR48692 had no effect (data not shown).}

Of the other behaviours examined, sniffing was significantly attenuated by chronic treatment with fluphenazine as was rearing (Table 8). However, neither of these behaviours was
Fig. 12. The effects of intra-nigral infusion of SR48692 on VCMs

Fig. 12. The effects of intra-nigral infusion of SR48692 on VCMs. Chronic treatment with fluphenazine decanoate induced VCMs. This behaviour was attenuated by intra-nigral infusion of SR48692 (0.25, 0.50, and 1.0 nmol/µl). Each bar represents the mean (+ S.E.M.) duration (s) of VCMs recorded over a 1-h period.

** Significantly different from chronic vehicle treatment, \( p < 0.01 \).
* Significantly different from vehicle + 0.25 nmol SR48692, \( p < 0.01 \).
* Significantly different from vehicle + 0.50 nmol SR48692, \( p < 0.05 \).
** Significantly different from vehicle + 0.50 nmol SR48692, \( p < 0.01 \).
* Significantly different from vehicle + 1.0 nmol SR48692, \( p < 0.05 \).
** Significantly different from vehicle + 1.0 nmol SR49692, \( p < 0.01 \).
# Significantly different from fluphenazine decanoate + 10% DMSO in saline, \( p < 0.05 \).
### Significantly different from fluphenazine decanoate + 10% DMSO in saline, \( p < 0.01 \).
Fig. 13. The effects of intra-pallidal infusion of SR48692 on VCMs

Intra-pallidal SR48692

Fig. 13. The effects of intra-pallidal infusion of SR48692 on VCMs. Chronic treatment with the neuroleptic fluphenazine decanoate induced the development of VCMs. This behaviour was suppressed by the 1.0 nmol/μl dose of SR48692 infused into the GP, but not by the 0.25 or 0.50 nmol/μl doses. Each bar represents the mean (+ S.E.M.) duration (s) of VCMs recorded over a 1-h period.

* Significantly different from chronic vehicle treatment, $p < 0.05$.
** Significantly different from chronic vehicle treatment, $p < 0.01$.
• Significantly different from vehicle + 0.25 nmol SR48692, $p < 0.05$.
** Significantly different from vehicle + 0.25 nmol SR48692, $p < 0.01$.
** Significantly different from vehicle + 0.50 nmol SR48692, $p < 0.01$.
• Significantly different from vehicle + 1.0 nmol SR48692, $p < 0.05$.
** Significantly different from vehicle + 1.0 nmol SR48692, $p < 0.01$.
### Significantly different from fluphenazine decanoate + 10% DMSO in saline, $p < 0.01$.
•• Significantly different from fluphenazine + 0.25 nmol SR48692, $p < 0.01$.
* Significantly different from fluphenazine + 0.50 nmol SR48692, $p < 0.05$. 

Vehicle (N = 6)
Fluphenazine (N = 7)
Table 8
The effects of intra-pallidal SR48692 on total time (s) engaged in rearing and sniffing.

<table>
<thead>
<tr>
<th>SR48692 (nmol/µl)</th>
<th>0.0</th>
<th>0.25</th>
<th>0.50</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>REARING</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>8.00 ± 6.38</td>
<td>6.89 ± 4.41</td>
<td>14.68 ± 4.86</td>
<td>7.47 ± 5.34</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>0.25 ± 0.25</td>
<td>2.00 ± 2.00</td>
<td>0.00 ± 0.00</td>
<td>1.56 ± 0.86</td>
</tr>
<tr>
<td>SNIFFING</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>365.15 ± 91.04</td>
<td>449.77 ± 97.67</td>
<td>357.66 ± 87.12</td>
<td>311.50 ± 87.66</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>199.69 ± 36.25</td>
<td>141.63 ± 26.63</td>
<td>204.58 ± 30.99</td>
<td>222.83 ± 60.58</td>
</tr>
</tbody>
</table>

Values represent the mean (± S.E.M.) duration of responses (s) scored over 10 blocks of 3 min. Rearing: $F_{1,11} = 11.06, p < 0.01$ (Fluphenazine main effect); $F_{3,33} = 0.36, p = \text{NS}$ (Treatment main effect); $F_{3,33} = 0.77, p = \text{NS}$ (Interaction effect). Sniffing: $F_{1,11} = 5.86, p < 0.05$ (Fluphenazine main effect); $F_{3,33} = 0.12, p = \text{NS}$ (Treatment main effect); $F_{3,33} = 1.83, p = \text{NS}$ (Interaction effect). N = 6, Vehicle; N = 7, Fluphenazine.
affected by treatment with SR48692. Neither locomotion nor grooming was affected by
treatment with either fluphenazine or SR48692 (Table 6).

4.4 Discussion

As expected, chronic treatment with neuroleptics resulted in the robust development of
VCMs in animals receiving FLU compared to those treated with VEH in all experiments. While
animals were used repeatedly within each experiment, different groups of animals were used for
each region and this may account for the disparity in response rates across experiments. As well,
while there was some disparity in guide cannula placement within both the striatum and the GP,
this was not found to have a consistent effect on behaviour.

Given the small volume and slow rate of infusion, it is likely that the diffusion of drug
was approximately one millimeter from the infusion site. It is therefore doubtful that the results
can be attributed to drug effects on brain structures other than those targeted. While it is possible
that dilution in 10% DMSO may have increased the diffusibility of the SR48692, this issue
remains controversial (Ziylan et al., 1988; Gupta et al., 2000; Park et al., 200; Van der Bijl et al.,
2001). Furthermore, drug treatment had no effect on behaviour in animals with incorrect cannula
placements.

SR48692 has high affinity for the NTS1, a receptor subtype that is present in the striatum,
the SNr, and, to a lesser degree, in the GP (Fassio et al., 2000). Thus, reduction of chronic
neuroleptic-induced VCMs following infusion of SR48692 into these regions is in keeping with
a key role of increased NT expression in both the striatum itself as well as its projection regions
in the emergence of TD. Whereas all 3 doses of SR48692 were effective within the striatum and
the SNr, in the GP, SR48692 blocked VCMs at only the highest dose administered (1.0 nmol/µl).

Although the striatum is thought to be important for the expression of oral dyskinesias
(Mackay, 1982; Pisa 1988; Kelley et al., 1989; Salamone et al., 1990) and NT administered into
the ventrolateral striatum elicits VCMs in neuroleptic-naïve animals (Stoessl, 1995), the effect of intra-striatal SR48692 on chronic neuroleptic-induced VCMs was partial as VCMs were not suppressed to control levels (Appendix I). This may mean that increased NT within the striatum following neuroleptic therapy plays a partial but incomplete role in the emergence of TD. On the other hand, the striatal NTS1 has been recently implicated in increased glutamate and GABA release in both the striatum and the GP (Ferraro et al., 1998). This could ultimately result in increased rather than decreased inhibition of the motor pathways, the opposite of what is thought to occur in TD. Alternatively, it is possible that either a higher dose of SR48692 or more selective infusion of SR48692 into the ventrolateral striatum might have suppressed VCMs to control levels.

The suppression of fluphenazine-induced VCMs in fluphenazine-treated animals following intra-nigral infusion of SR48692 is in keeping with the known increase in striatal NT following treatment with haloperidol (Deutch & Zahm, 1992; Merchant et al. 1992, 1994; Chartoff et al., 1999) and the small increase in expression of NT in the striatonigral projection (Goedert et al., 1985). While all three doses of intra-SNr SR48692 significantly reduced the duration of VCMs, none was able to suppress the behaviour to control levels (Appendix II). Recent evidence has indicated a role for NTS1s in the release of both GABA and glutamate in the SNr (Ferraro et al., 2001). Intra-nigral infusion of NT increased glutamate release and decreased GABA release in the SNr (Ferraro et al., 2001), which could increase inhibition of the motor pathways. However, it also decreased ventral thalamic GABA release (Ferraro et al., 2001), which, in turn, could disinhibit the motor pathways. Thus, while it seems that NTS1s play a role in the modulation of neurotransmitter release at the level of the SNr, the behavioural result of this modulation remains to be clarified. Our findings suggest that increased expression of
nigral NT and NTS1s following chronic neuroleptic therapy may play a role in the 
extrapyramidal side effects of this treatment.

The intra-pallidal infusion of SR48692 also suppressed VCMs to control levels at the 
highest dose administered (1.0 nmol/μl), while it was ineffective at lower doses (Appendix III). 
The failure of SR48692 to suppress VCMs at lower doses may be due to the limited expression 
of NTS1 in the GP (Fassio et al., 2000), which has a comparatively higher level of NTS2 
(Walker et al., 1998). In addition, a recent study has suggested that, in Chinese hamster ovary 
cells synthesizing rat NTS2, SR48692 may unexpectedly have partial agonist properties (Vincent 
et al., 1999). Nevertheless, the full suppression of neuroleptic-induced VCMs at 1.0 nmol/μl 
suggests that increased NT expression in both the indirect striatopallidal projection (Brog & 
Zahm, 1996), as well as the direct striatonigral pathway (Goedert et al., 1985), is important in the 
pathogenesis of TD.

The rodent model of TD used in these experiments, vacuous chewing movements 
(VCMs) induced by chronic neuroleptic therapy, remains controversial. Some researchers have 
argued that VCMs are more analogous to Parkinsonian tremor (Jicha & Salamone, 1991) or 
acute dystonia (Rupniak et al., 1985) than to TD. This controversy notwithstanding, there are 
similarities between chronic neuroleptic-induced VCMs and TD which support its use as a 
putative model of TD: VCMs develop along similar time lines to TD (Glenthoj et al., 1990); 
VCMs persist following withdrawal from medication (Waddington et al., 1983; Ellison, 1991); 
and they are suppressed by treatment with dopamine receptor antagonists (Stoessl et al., 1989) or 
by dopamine depletion (Diana et al., 1992).

The development of VCMs along similar time lines to TD is an important issue in the 
evaluation of this rodent model. While some studies have found the emergence of VCMs early 
in the course of neuroleptic treatment, other studies have revealed these movements to be
qualitatively and pharmacologically different from those VCMs which emerge following long
term treatment (Andreassen & Jorgensen, 1995; Egan et al., 1996). Thus, in our studies, the
animals were not observed for development of VCMs during the initial fluphenazine treatment
period.

The quantification of VCMs is reported in different ways by groups using this model.
While a number of groups use frequency of VCMs as a measure, our lab scores the total duration
of VCMs over the testing period (Stoessl et al., 1989, 1993; Stoessl & Szczutkowski, 1991;
Stoessl, 1995, 1996; Van Kampen & Stoessl, 2000; McCormick & Stoessl, 2002) and was
carried out by a single rater. Given the tendency of VCMs to occur in bursts, we have found that
total duration of VCMs is more likely accurately reflect the behaviour than total frequency.

The results of these experiments may be clinically relevant, because even though newer
atypical antipsychotic agents have emerged with a lower incidence of TD and other
extrapyramidal side effects, older generation antipsychotics are still widely used in the treatment
of schizophrenia. These findings suggest that increased expression of NT within the striatum as
well as striatal output pathways may contribute to the development of TD. Thus, treatments that
reduce or prevent the effects of increased NT expression may help to manage this disabling
condition.
CHAPTER V Discussion and Conclusion

5.1 Relevance of Findings

In accordance with previous studies, the findings here confirm that chronic treatment with the typical neuroleptic fluphenazine decanoate versus its vehicle, sesame oil, leads to the robust development of VCMs. We did not quantify the duration of VCMs during the initial phase of neuroleptic treatment; hence, we are unable to address the issue of early- versus late-developing VCMs. However, as previous studies have shown distinctive qualitative and pharmacological attributes in early- versus late-developing VCMs (Andreassen & Jorgensen, 1995; Egan et al., 1996), we feel that the issue of whether chronic neuroleptic-induced VCMs develop along a similar time course as TD in humans has been sufficiently dealt with. Thus, given the period of chronic neuroleptic administration used in our studies, we regard the behaviour elicited in our animals as tardive in nature. In addition, while the duration of VCMs elicited under control conditions in the fluphenazine-treated animals varies between experiments, the magnitude of chronic fluphenazine-induced VCMs in all studies was substantial.

Furthermore, as there is variability in the development of TD in humans, the individual inter-animal differences found in our studies support its use as a putative model of TD.

Our studies furthermore demonstrate that infusion of opioid or non-opioid peptide receptor antagonists into the striatum, the SNr, or the GP suppresses the manifestation of neuroleptic-induced VCMs. Previous reports have described increased DYN mRNA levels in the striatonigral projection (Egan et al., 1994, 1996), increased ENK mRNA in the striatopallidal projection (Hong et al., 1978; Tang et al., 1983; Auchus & Pickel, 1992), and increased striatal NT mRNA levels as well as altered striatal and nigral NT receptor binding (Uhl & Kuhar, 1984; Bolden-Watson et al., 1993) following chronic neuroleptic treatment. This evidence together with our finding that blockade of the effects of increased neuropeptide levels by receptor
antagonists suggests a prominent role for increased levels of neuropeptide expression in the emergence of TD.

The elaboration of the role played by neuropeptides in the central nervous system is ongoing. While their co-localization in neurons expressing classical neurotransmitters suggests a modulatory role, the functional significance of this modulatory influence is not yet fully elucidated. The manner in which opioid and non-opioid peptides differentially affect the release of transmitters such as GABA and glutamate within specific nuclei of the basal ganglia (Maneuf et al., 1994, 1995; Ferraro et al., 1998, 2001) helps to clarify the impact that increased levels of these neuropeptides may have on the activity of the projection pathways. Thus, the inhibition of glutamate release from the subthalamonigral projection by DYN (Maneuf et al., 1995) and the inhibitory effect of ENK on the release of GABA in the GP (Maneuf et al., 1994) could both disinhibit the thalamocortical projection leading to dyskinesia. Our findings support this proposal since blockade of κ-, δ-, or μ-opioid receptors by specific antagonists attenuates the manifestation of dyskinetic movement. However, the modulation of GABA and glutamate release by NT and its impact on motor function needs further clarification. Increased GABA and glutamate release in both the striatum and GP as a result of intra-striatal infusion of NT (Ferraro et al., 1998) could inhibit the thalamocortical projection, thereby leading to hypokinetic disorders. Moreover, the finding that intra-nigral infusion of NT increases glutamate and decreases GABA release in the SNr (Ferraro et al., 2001) seems to support a role for NT in the emergence of hypokinetic movement disorders. However, intra-nigral NT infusion also decreased GABA release in the ventral thalamus, thereby suggesting disinhibition of the thalamocortical projection. Furthermore, our data suggest that the outcome of modulation of glutamate and GABA release in the basal ganglia by NT might ultimately act to disinhibit
thalamocortical outflow, as blockade of NTS1s in the striatum, GP and SNr suppresses the emergence of dyskinesia.

In so far as increased levels of mRNA expression may be indicators of increased neuronal activity, increased neuropeptide expression levels in projection regions of both the direct striatonigral and indirect striatopallidal pathways suggest altered activity in both pathways concurrently. This supports the proposal that dyskinesia is due to the altered balance and pattern of activity in both pathways rather than disrupted activity in one or the other, as the classical model of motor function would suggest. Furthermore, our findings support our hypothesis that altered activity in both pathways concomitantly is necessary for the expression of dyskinesia and that neutralizing this disrupted activity in either pathway will result in the suppression of dykinesia. Through these experiments, we attempt to delineate which, if any, projection pathway plays a more prominent role in the emergence of dyskinesia. However, while our results support the proposal that modified activity in both pathways contributes to dyskinesia, the findings are unable to clarify the relative contributions of each to the development of dyskinesia, since the pharmacological manipulation of receptor function in either projection pathway attenuates VCMs in all studies. It is possible, therefore, that both projection pathways play a prominent role in the emergence of dyskinesia and that the pattern of activity within each pathway makes an equivalent contribution to the development of motor function and dysfunction.

The finding that both pathways seem to be implicated in the emergence of dykinesia allows some seemingly contradictory evidence to be incorporated into a more complex model of motor circuitry. The co-occurrence of neuroleptic-induced parkinsonism and TD is more understandable if the direct striatonigral and the indirect striatopallidal pathways are both implicated in emerging motor disorders. The degree to which the balance and pattern of activity changes over the course of chronic neuroleptic treatment may allow for the co-occurrence of
these disorders which have traditionally been supposed to occur through opposing mechanisms. Furthermore, if, as our findings suggest, suppression of the effects of abnormal activity in either projection pathway alleviates the expression of dyskinesia, then the effect of pallidotomy on LID, hemi-ballismus, and TD may not seem as paradoxical as once thought.

5.2 Future Directions

Our findings have indicated some behavioural correlates of increased opioid and non-opioid peptide expression in the direct and indirect projection pathways of the basal ganglia. However, the suppression of neuroleptic-induced VCMs by all the compounds tested leaves questions unanswered and prompts possibilities for future investigation.

As intra-pallidal infusion of naltrindole (NTI) suppressed neuroleptic-induced VCMs at all three doses tested, the dose-response curve for NTI is incomplete. Therefore, the effect of intra-pallidal infusion of NTI at 0.05 and 0.1 nmol/μl doses should be tested in order to establish the lowest effective dose for the suppression of neuroleptic-induced VCMs.

While the distribution of κ-, δ-, and μ-opioid receptors differs widely in the basal ganglia, it may be interesting to examine whether their specific antagonists infused into different nuclei have an effect on neuroleptic-induced VCMs. Thus, experiments investigating the effects of intra-nigral infusion of CTOP or intra-nigral infusion of NTI on neuroleptic induced VCMs can be conducted, as both δ-opioid (Tempel & Zukin, 1987) and μ-opioid (German et al., 1993; Unterwald et al., 1998) receptors are present in the SNr.

Further examination of the contribution of increased levels of DYN expression should also be undertaken. As the effect of intra-nigral nor-BNI infusion on neuroleptic-induced VCMs was incomplete with only the highest dose suppressing VCMs, increased expression of DYN may play a more minor role in the emergence of TD compared to the effects of increased ENK. Examination of the effects of intra-pallidal infusion of CTOP or NTI concurrent with intra-nigral
infusion of nor-BNI could be performed. The combined effects on neuroleptic-induced VCMs of κ- and δ- or κ- and μ-opioid receptor antagonists may help to further clarify this issue.

In an effort to further clarify the importance of increased levels of neuropeptide expression in dykinesia, the effect of neuropeptide level increases in neuroleptic-naïve animals could be investigated. Neuropeptides are degraded by neuropeptidases; therefore, alteration of peptidase activity levels could result in changes in neuropeptide catabolism. In the caudate-putamen, the activity of aminopeptidase N, neutral endopeptidase 24.11, and metalloendopeptidase 24.15 are all decreased following haloperidol administration (Waters et al., 1996), which could contribute to increases in ENK expression. Since potent inhibitors of aminopeptidase N and neutral endopeptidase 24.11 exist (Roques & Noble, 1995), the effect of these compounds on behaviour in neuroleptic-naïve animals could be examined. Infusion of the systemically active peptidase inhibitor, N- [(R,S)-2-benzyl-3 [(S) (2-amino-4-methylthio) butyl dithio] -1-oxopropyl] –L-phenylalanine benzyl ester (RB101) (Noble et al., 1992) into the SNr or the GP of neuroleptic-naïve animals may elicit VCMs, thereby helping to further clarify the extent of ENK's role in the emergence of dyskinesia.

More experiments will need to be conducted to further clarify the role of increased NT expression levels in neuroleptic-induced VCMs. Both intra-striatal and intra-nigral infusion of SR48692 significantly attenuated the expression of VCMs, but were unable to suppress VCMs to control levels. Thus, experiments that examine the effect on neuroleptic-induced VCMs of a higher dose of SR48692 (2.0 nmol/μl) could be conducted.

As the ventrolateral striatum has been implicated in oral dyskinesias (Mackay, 1982; Pisa 1988; Kelley et al., 1989; Salamone et al., 1990), it would be instructive to examine a more localized infusion of SR48692. Therefore, a more selective infusion of SR48692 into the
ventrolateral striatum could be conducted with the expectation that neuroleptic-induced VCMs will be suppressed to control levels by this manipulation.

Lastly, the effect of SR48692 infusion into the GP was incomplete, as suppression of neuroleptic-induced VCMs was evident only at the highest dose administered. This is possibly due to the relatively low level of NTS1 expression in the GP (Fassio et al., 2000) compared to that of NT receptor 2 (NTS2) (Walker et al. 1998). As a NT receptor antagonist exists which has affinity for both receptor subtypes (Gully et al., 1997), an investigation into the effect of intra-pallidal infusion of SR142948A could be undertaken to evaluate if the actions of NT at NTS2s contribute to neuroleptic-induced VCMs.

A final exploration into the contribution of increased NT expression levels in the striatopallidal projection in the emergence of dyskinesia could look at the combined effect of intra-pallidal and intra-nigral infusion of SR48692. Thus, if infusion into both the GP and the SNr is effective at suppressing neuroleptic-induced VCMs at lower doses than our findings have shown, the relative contributions of the striatonigral and striatopallidal pathways may be further delineated.

5.3 Conclusion

To date, there is no effective treatment for the management of TD. Although atypical antipsychotic drugs are less prone to elicit extrapyramidal side effects, these drugs are not a satisfactory solution with regard to TD. Thus, TD has been found in a patient treated with quetiapine (Ghaemi & Ko, 2001) and switching the treatment regime to an atypical antipsychotic has had little beneficial effect in some patients with TD (Modestin et al., 2000). Furthermore, the potential risk for developing TD increases with age with some reporting a prevalence rate of 67% in a population of geriatric in-patients (Saltz et al. 1991). The estimated risk also increases with length of neuroleptic exposure from a rate of 31.8% with 5 years’ exposure to 68.4% with
25 years' exposure (Glazer, 2000). Hence, the need for strategies which prevent its emergence or manage its presence still remains.

Our findings suggest that, in order to prevent the manifestation of neuroleptic-induced TD, therapy involving concomitant administration of peptide antagonists might present a possible solution. Since peptides have low penetrability across the blood-brain barrier, therapies using peptidergic agents have, until recently, been problematic. However, the development of a number of non-peptide antagonists, which readily cross the blood-brain barrier, has greatly increased the feasibility of modifying the effects of neuropeptide activity in a therapeutic manner. As well, since neuropeptides have modulatory actions, it is possible that blockade of their receptors may have less dramatic consequences than seems to be the case for amino acid transmission (Hökfelt et al., 2000). Moreover, as increased neuropeptide release seems to be associated with abnormal conditions in some systems, then neuropeptide antagonists may only affect those systems with abnormally increased peptide release (Hökfelt et al., 2000). The end result could be the emergence of fewer side effects.

As opioid and non-opioid peptides often have more than one receptor subtype with different functional profiles, this necessitates the development of agents with selective actions at these receptor sub-types. Since the localization of neuropeptide receptors is widespread in the central nervous system, antagonists with highly selective receptor subtype affinity are crucial in order to ensure that these agents will have the desired effects. Thus, the specificity of these compounds for their receptor subtypes may also limit their side effects, thereby improving their viability in treatment regimes.

Since a number of these compounds exist that either reduce or prevent the effects of increased neuropeptide levels in the basal ganglia, it may be possible to combine their use with antipsychotic medication in order to better manage TD. Thus, while investigation into the
mechanisms responsible for TD continues, enhanced strategies for its management may provide some relief to the problem.
BIBLIOGRAPHY


Coyle J.T., Schwarcz R. (1976) Lesion of striatal neurones with kainic acid provides a model for Huntington’s chorea. *Nature* 263, 244-246.


denervation and L-DOPA therapy on the expression of glutamic acid decarboxylase messenger RNA in the pallidum. *Neurology, 47*, 219-224.


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APPENDIX I Intra-striatal SR48692 Pairwise Comparison Values

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SNK values performed post repeated measures 2-way ANOVA on VCM duration following intra-striatal infusion of SR48692 ($F_{1,14} = 66.42, p < 0.001$, FLUPHENAZINE main effect; $F_{3,42} = 8.38, p < 0.001$, TREATMENT main effect; $F_{3,42} = 7.24, p < 0.001$, FLUPHENAZINE x TREATMENT interaction effect). $N = 8$, Vehicle; $N = 8$, Fluphenazine. *$p < 0.05$. **$p < 0.01$. 
## APPENDIX II Intra-nigral SR48692 Pairwise Comparison Values

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SNK values performed post repeated measures 2-way ANOVA on VCM duration following intra-striatal infusion of SR48692 (\( F_{1,11} = 16.91, \ p < 0.01, \) FLUPHENAZINE main effect; \( F_{3,33} = 2.89, \ p < 0.05, \) TREATMENT main effect; \( F_{3,33} = 2.93, \ p < 0.05, \) FLUPHENAZINE x TREATMENT interaction effect). N = 6, Vehicle; N = 7, Fluphenazine. * \( p < 0.05. \) ** \( p < 0.01. \)
APPENDIX III Intra-pallidal SR48692 Pairwise Comparison Values

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SNK values performed post repeated measures 2-way ANOVA on VCM duration following intra-striatal infusion of SR48692 (F_{1,11} = 18.10, p < 0.01, FLUPHENAZINE main effect; F_{3,33} = 3.16, p < 0.05, TREATMENT main effect; F_{3,33} = 2.20, p = NS, FLUPHENAZINE x TREATMENT interaction effect). N = 6, Vehicle; N = 7, Fluphenazine. * p < 0.05. ** p < 0.01.