THE ROLE OF SUBSTANCE P IN EARLY EXPERIMENTAL PARKINSON’S DISEASE

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November 2008

A thesis submitted to the University of Adelaide in partial fulfilment of the requirements for the degree of Doctor of Philosophy
CHAPTER 5:
THE EFFECTS OF CAPTOPRIL IN THE INTRASTRIATAL
6-OHDA MODEL OF EARLY PARKINSON’S DISEASE
5.1 Introduction

In the previous chapters, it was shown that the PD neurotoxin, 6-OHDA, increased SP production both in vitro and in vivo, and that elevated SP content in vitro resulted in greater LDH production, a marker of cell death. Thus increased amounts of SP may contribute to 6-OHDA induced cell death. The widely used angiotensin-converting enzyme (ACE) inhibitor, Captopril, prevents the degradation of SP because ACE is one of two enzymes involved in the catabolism of SP. Thus Captopril usage may exacerbate dopaminergic cell death in early PD.

Captopril is widely used clinically for the treatment of hypertension and chronic heart failure due to its ability to inhibit the formation of angiotensin II, a potent vasoconstrictor (Godinez-Hernandez et al., 2006). Apart from being integral to the renin-angiotensin system, angiotensin II is also found within the striatum and SN, where ACE is also present in high levels (Jenkins et al., 1997; Arinami et al., 1996). As previously mentioned, ACE is involved in the degradation of the neuropeptide, SP, which as previously indicated is in high concentrations in the SN. Interestingly, chronic treatment with Captopril has previously been shown to be beneficial in both experimental and human Parkinson’s disease (Kurosaki et al., 2005; Lopez-Real et al., 2005; Munoz et al., 2006; Reardon et al., 2000). Captopril treatment increases DA synthesis and release, possibly by inhibiting the degradation of SP; SP binds to NK1 receptors on dopaminergic neurons in the SN to stimulate DA production (Jenkins et al., 1997; Jenkins et al., 1999). ROS are also involved in the signalling of angiotensin II, and as such ACE inhibitors may act as an antioxidant. These antioxidant properties may further contribute to the attenuation of dopaminergic cell loss and subsequent loss of striatal DA derivatives, DOPAC and HVA in both 6-OHDA and MPTP models of PD (Kurosaki et al., 2005;
Lopez-Real et al., 2005; Munoz et al., 2006). However, these beneficial effects of ACE inhibitors were seen in experimental models of PD that replicated the late stages of the disease and moderately severe human PD, when dopaminergic cell death is almost complete. It should also be noted that in the late stages of PD, there is a decrease in SP content within the SN (De Ceballos and Lopez-Lozano, 1999; Mauborgne et al., 1983; Nisbet et al., 1995; Tenovuo et al., 1984). Thus the effects of ACE inhibitors may be strongly dependent on the stage of disease progression.

The effects of ACE inhibitors such as Captopril in the early stages of the disease have never been investigated. In the 6-OHDA intrastriatal model of PD, which replicates the early stages of PD, degeneration of dopaminergic neurons is slow and progressive as is observed in human PD. Also in this model, our earlier results have shown that SP expression was elevated in the striatum and was located within surviving dopamine neurons. Accordingly, the present study examined the effects of chronic Captopril treatment on functional and histological outcome in the intrastriatal model of early PD, with a particular focus on motor function, dopaminergic degeneration, SP expression, inflammatory response and BBB dysfunction.

5.2 Study Design

Animals (n=32) were randomly assigned to either vehicle or Captopril treatment groups. Animals were then given subcutaneous injections of Captopril (Sigma; 5mg/kg) or equal volume of saline vehicle for 7 days prior to, and 21 days after, intrastriatal 6-OHDA injections. Captopril (5mg/ml) was dissolved in 0.9% saline and stored at 4°C for a maximum of 4 days. All animals were subject to intrastriatal 6-OHDA lesions (as
described in Chapter 2.3.2) and then randomly assigned to be sacrificed at day 3, 7 or 21 post-lesion. Briefly, animals received stereotaxic intrastriatal injections of 6-OHDA (2 × 2 μl; 5 μg/μL). The 21-day animals were assessed for functional outcome (as described in Chapter 2.4.1-2.4.7). Striatal and SN sections from each animal were then assessed histological outcome (as described in Chapter 2.5.2-2.5.7). Sham animals (n=5), which underwent the surgical procedures but did not receive 6-OHDA, were used as a control for both functional and histological outcome.

5.2.1 Functional Outcome

The functional outcome of both Captopril and vehicle animals was assessed on days 3, 7, 10, 14, 17 and 21 following intrastriatal 6-OHDA lesions. Motor function was assessed using the rotarod, stepping tests and bilateral asymmetry test. A modified neurological severity score (mNSS) was used to assess neurological outcome and the open field test to determine behavioural outcome. Lesion size was estimated using the rotometer on days 7 and 14 post-lesion. All functional tests are described in detail in chapter 2.4.

5.2.2 Histological Analysis

At either day 3, 7 or 21 post-lesion, animals were deeply anaesthetised with Halothane and perfused fixed with 10% buffered formalin, as described in Chapter 2.3.4. Brains were then removed and processed for immunocytochemistry. Sections of the striatum and the SN were stained for TH, H&E, SP, ED-1, GFAP and albumin, and then assessed using light microscopy.
5.2.3 Statistical Analysis

All parametric data were analysed using an ANOVA followed by Bonferroni post-tests, and are displayed as mean ± SEM. Neurological and behavioural outcome are displayed as the median and therefore data were analysed using the Kruskal Wallis non-parametric ANOVA followed by Dunn’s multiple comparisons test.

5.3 Results

5.3.1 Functional Outcome

_Motor Function - Rotarod_

Sham animals had normal motor function as assessed by the rotarod (Figure 5.1), with all animals completing the 2-minute task. Intrastriatal injections of 6-OHDA resulted in a highly significant ($p < 0.001$) decline in motor function that persisted throughout the assessment period, with animals completing an average $76 ± 4$ seconds on the rotarod. Chronic Captopril treatment further exacerbated motor deficits, especially on day 3 post-lesion when these animals were significantly worse ($51 ± 6$ seconds; $p < 0.001$) than vehicle treated animals. Despite the improvement in motor function by day 7, Captopril treated animals did not regain equal function to vehicle treated animals at any day post-lesion. Furthermore, neither of the 6-OHDA groups were able to significantly improve on the rotarod task, consistent with these animals having a sensorimotor learning deficit (Ogura et al., 2005).
**Figure 5.1:** Chronic Captopril treatment – Motor function as assessed by the rotarod.

Sham animals (black) had normal motor function. Intrastriatal 6-OHDA lesions induced significant motor deficits throughout the assessment period, with vehicle treated animals (orange) demonstrating poor motor function compared to shams. Chronic Captopril treatment further exacerbated this motor deficit, with Captopril+6-OHDA animals (aqua) displaying significantly worse motor function than vehicle treated animals on day 3 post-lesion. (*** denotes \( p < 0.001 \) versus vehicle) (n=6 / 6-OHDA groups; n =5/Sham group).
Motor Function - Stepping Tests

Initiation Time

Sham animals had normal initiation of ipsilateral and contralateral forepaw movement as assessed by the stepping test, with all animals moving both forepaws in approximately one second (Figure 5.2). 6-OHDA striatal lesions significantly delayed initiation of forepaw movement over the entire assessment period ($p < 0.001$). Although neither of the 6-OHDA groups showed much improvement in this task, the vehicle treated group improved from an average of $7.5 \pm 1.4$ seconds to $5.0 \pm 1.7$ seconds in their ipsilateral forepaw by day 21 post-lesion, whereas at this time Captopril treated animals remained at the maximum initiation time of $10 \pm 0$ seconds. Accordingly, Captopril treated animals had a longer delay in initiation than vehicle treated animals, which was significant on day 21 post-lesion ($p < 0.05$). In contrast, on the contralateral side, vehicle treated and Captopril treated animals did not record significantly different delays in initiation, although Captopril treated animals had a trend toward longer initiation times on days 7, 10, 17 and 21 post-lesion.

Stepping Time

Sham animals quickly learnt the stepping test, taking on average $6.1 \pm 0.3$ seconds to traverse the ramp (Figure 5.3). However, 6-OHDA striatal lesions resulted in longer stepping times. This was particularly apparent on day 3 post-lesion, when vehicle treated animals recorded a stepping time of $28.0 \pm 7.1$ seconds, which was significantly longer than sham animals ($p < 0.001$). Treatment with Captopril further exacerbated motor deficits as they took $59.5 \pm 0.5$ seconds to walk along the 60 cm ramp. Thus these animals were significantly worse than vehicle treated animals at day 3 post-lesion ($p < 0.001$).
Figure 5.2: Chronic Captopril treatment – Initiation of movement as assessed by the stepping tests.

Sham animals (black) had normal initiation of movement in both their ipsilateral (A) and contralateral (B) forepaws. In contrast, 6-OHDA lesioned animals had a significant delay in initiation, as vehicle treated animals (orange) recorded longer initiation times in both forepaws throughout the assessment period compared to shams. Captopril treated animals (aqua) had a further delay in their initiation of movement, particularly in their ipsilateral forepaw where these animals recorded a significantly longer delay in initiation on day 21 post-lesion than vehicle treated animals. (* denotes $p < 0.05$ versus vehicle) (n=5/sham; n=6 / 6-OHDA groups).
A. **Ipsilateral Initiation Time**

![Graph showing time (in seconds) on the y-axis and day post-lesion on the x-axis for different groups: Sham, Captopril + 6-OHDA, Capt Vehicle + 6-OHDA.]

B. **Contralateral Initiation Time**

![Graph showing time (in seconds) on the y-axis and day post-lesion on the x-axis for different groups: Sham, Captopril + 6-OHDA, Capt Vehicle + 6-OHDA.]

*Note: The graph shows significant differences between groups indicated by asterisks.*
Sham animals (black) had a quick, constant stepping time throughout the assessment period. Conversely, 6-OHDA striatal lesioned animals took longer to traverse the ramp, with vehicle treated animals (orange) taking significantly longer than shams on day 3 post-lesion. These animals had improved by day 7 but did not return to sham levels until day 14 post-lesion. This motor deficit was further exacerbated in Captopril treated animals (aqua), which recorded a significantly longer stepping time than vehicle treated animals on day 3. Despite improvement during the assessment period, Captopril treated animals always recorded a longer stepping time than vehicle treated animals. (** denotes $p < 0.001$ versus vehicle) (n=5/sham; n=6 / 6-OHDA groups).
Throughout the assessment period both 6-OHDA groups improved, however vehicle treated animals returned to sham levels by day 14, whereas Captopril treated animals always recorded a slightly longer stepping time than vehicle treated animals and did not return to sham levels during the assessment period.

**Step Length**

Sham animals had a relatively constant step length averaging 9.6 ± 0.3 cms for the 21 day assessment period (Figure 5.4). In contrast, 6-OHDA striatal lesioned animals had a reduced step length on day 3 post-lesion, with vehicle treated animals recording a step length of 6.9 ± 0.3 cms, although this was not significantly shorter than shams. However, Captopril treated animals had a further reduction in step length on day 3, recording a step length of only 3.3 ± 0.4 cms, which was significantly shorter than vehicles (p < 0.01) and sham animals (p < 0.001). Furthermore, these animals did not return to sham levels until day 14 post-lesion, unlike vehicle treated animals that had improved to this level by day 7 post-lesion. Overall, these stepping tests results are consistent with rotarod motor function, where Captopril treated animals had especially poor function during the first week, and although improved, did not regain equal motor function to vehicle treated animals.

**Adjusting Steps**

Sham animals had normal movement in their contralateral forepaw in both the forehand and backhand direction (Figure 5.5), performing on average 6.1 ± 0.8 and 6.9 ± 0.4 adjusting steps, respectively. The forehand direction is the most affected following intrastriatal 6-OHDA injections, producing a significant (p < 0.001) lack of movement.
Figure 5.4: Chronic Captopril treatment – Step length as assessed by the stepping tests.

Sham animals (black) recorded a relatively constant step length throughout the 21-day assessment period. Following 6-OHDA striatal lesions, vehicle treated animals (orange) recorded a non-significant decline in step length on day 3 then returned to sham levels by day 7 post-lesion. However, Captopril treatment in 6-OHDA animals exacerbated motor deficits so that these animals recorded a significantly shorter step length than vehicle treated animals on day 3 post-lesion, and despite improvement did not return to sham levels until day 14 post-lesion. (**) denotes p < 0.01 versus vehicle) (n=5/sham; n=6 / 6-OHDA groups).
Figure 5.5: Chronic Captopril treatment – Contralateral forepaw adjusting steps as assessed by the stepping tests.

Sham animals (black) did not display akinesia features as they could perform adjusting steps with their contralateral forepaw in both the forehand (A) and backhand (B) directions. In contrast, 6-OHDA lesioned animals showed some akinesia features as vehicle treated animals (orange) performed less contralateral adjusting steps in the forehand direction than shams throughout the entire assessment period. Captopril treatment (aqua) resulted in a further loss of movement with these animals only able to perform a few steps. Thus Captopril treated animals performed significantly less forehand adjusting steps than vehicle treated animals on days 7 and 21 post-lesion. In the backhand direction, vehicle treated animals only had a slight decline in function compared to shams. Captopril treatment further exacerbated this loss of function, with animals displaying a gradual decline in function during the assessment period. (* denotes $p < 0.05$; ** denotes $p < 0.01$ versus vehicle) (n=5/sham; n=6 / 6-OHDA groups).
A. **Forehand Adjusting Steps**

![Graph showing the number of contralateral steps over days post-lesion for different treatment groups.]

B. **Backhand Adjusting Steps**

![Graph showing the number of contralateral steps over days post-lesion for different treatment groups.]

**Legend:**
- Sham
- Captopril + 6-OHDA
- Capt Vehicle + 6-OHDA

**Note:**
- **Star** indicates statistical significance at the 0.05 level.
- **Double star** indicates statistical significance at the 0.01 level.
with animals dragging their contralateral paw across some of the surface. As such, vehicle treated animals recorded on average only 2.6 ± 0.2 forehand adjusting steps during the 3-week period. Similar to other functional tests, Captopril treatment further exacerbated the loss of movement, with these animals performing an average of only 0.3 ± 0.3 forehand adjusting steps for the assessment period. Accordingly, Captopril treated animals did significantly less forehand adjusting steps with their contralateral paw than vehicle treated animals on days 7 and 21 post-lesion (0.01< p < 0.05).

Although all groups began with similar number of backhand adjusting steps on day 3 post-lesion, the ability of 6-OHDA lesioned animals to perform adjusting steps decreased as time progressed. Those administered Captopril demonstrated an even greater loss of movement. Despite the small loss of forepaw movement, vehicle treated animals did not perform significantly less steps than sham animals. In contrast, Captopril treated animals had a greater loss of forepaw movement than vehicle treated animals performing significantly less steps than shams (p < 0.01). Although, Captopril treated animals performed less backhand adjusting steps than vehicle treated animals, there were no significant differences at any individual time point between these groups due to the large within group variation in the Captopril group.

**Motor Outcome – Bilateral Asymmetry Test**

Sham animals had normal sensorimotor function in their forepaws as they could sense and remove the tape within 2 to 5 secs (Figure 5.6). In contrast, 6-OHDA striatal lesioned animals showed a gradual decline in sensorimotor function during the assessment period with vehicle treated animals recording longer latencies in both forepaws than shams at
**Figure 5.6: Chronic Captopril treatment – Ipsilateral and contralateral latencies as assessed by the bilateral asymmetry test.**

Sham animals (black) recorded normal sensorimotor function in both their ipsilateral (A) and contralateral (B) forepaws. Following 6-OHDA striatal lesions, a gradual loss of sensorimotor function was observed during the assessment period in both the ipsilateral and contralateral forepaws of vehicle treated animals (orange). Sensorimotor function was slightly worsened by chronic Captopril treatment, as Captopril treated animals (aqua) tended to have longer latency scores than vehicle treated animals. (n=5/sham; n=6 / 6-OHDA groups).
A. **Ipsilateral Latency**

![Graph showing Ipsilateral Latency over Day Post-Lesion. The graph displays data for Sham, Captopril + 6-OHDA, Capt Vehicle + 6-OHDA, and Sham with 6-OHDA conditions.](image)

B. **Contralateral Latency**

![Graph showing Contralateral Latency over Day Post-Lesion. The graph displays data for Sham, Captopril + 6-OHDA, Vehicle + 6-OHDA, and Capt Vehicle + 6-OHDA conditions.](image)
each assessment point, with significantly longer ipsilateral latency times on day 21 (p < 0.05) and contralateral latency times on day 17 post-lesion (p < 0.05) than shams. Captopril treated animals showed a similar trend for loss of sensorimotor function in their forepaws, with a significantly longer ipsilateral and contralateral latency than shams on day 17 post-lesion (p <0.05 and p < 0.001, respectively). In general Captopril treated animals recorded longer latency scores than vehicle treated animals, however the within group variability, particularly in the Captopril group prevented a statistically significant difference between the groups.

**Neurological Outcome - mNSS**

Sham animals had normal neurological function as assessed by the mNSS (Figure 5.7), with all animals scoring a median ranking of 0 on every assessment day. Although, 6-OHDA striatal lesions worsened neurological outcome, vehicle treated animals did not have significantly worse neurological function than sham animals, scoring a median ranking of no more than 3 on the mNSS. However, Captopril treatment exacerbated neurological deficits, with Captopril treated animals scoring a median mNSS score between 3.5 and 5 on each assessment day. Consequently, Captopril treated animals had a significantly (p < 0.001) worse neurological outcome than sham animals.

**Behavioural Outcome – Open Field**

Sham animals had normal exploratory behaviour within the open field throughout the improvement in activity level recording 8.5 on day 7 post-lesion. However, these animals did not display habituation to the task until day 17 post-lesion. Similarly, Captopril
Figure 5.7: Chronic Captopril treatment – Neurological outcome as assessed by the modified Neurological Severity Score.

Sham animals (black) had normal neurological function as assessed using the mNSS. In contrast, 6-OHDA lesioned animals recorded points on the mNSS at each day post-lesion with vehicle treated animals (orange) demonstrating mild neurological deficits. However, Captopril treated animals (aqua) recorded moderate neurological deficits on the mNSS during the first week post-lesion, and although they showed slight improvement always recorded a higher mNSS than vehicle treated animals. (n=5/sham; n=6 / 6-OHDA groups).
Figure 5.8: Chronic Captopril treatment – Behavioural outcome as assessed by the open field task.

Sham animals (black) demonstrated normal behaviour during the assessment period, with high levels of spontaneous exploration and therefore activity ranking on day 3 post-lesion followed by a decline in activity as they became habituated to the open field. In contrast, 6-OHDA striatal lesions produced atypical behaviour, with vehicle treated animals (orange) recording a decline in activity on day 3 post-lesion, before a slight improvement in activity. These animals did not become habituated to the task until day 17 post-lesion. Captopril treated animals (aqua) also had a decline in activity on day 7 then improvement. However, Captopril treated animals tended to have a greater activity level than shams and vehicle treated animals from day 7 onwards. Captopril treated animals also did not become habituated to the task until day 17 post-lesion. (n=5/shams; n=6 / 6-OHDA groups).
treated animals also recorded a reduction in activity level on day 3 post-lesion with a ranking of 5.5 before recording the maximum activity ranking of 10 on day 7 post-lesion. The activity ranking of these animals remained greater than both shams and vehicle treated animals throughout the assessment period. Captopril treated animals did not record a decline in activity until day 17 post-lesion, when they scored an activity ranking of 3 due to habituation to the task. Despite this, no significant difference in activity ranking was observed between any groups during the assessment period.

**Estimation of Lesion Size - Rotometer**

The rotometer test is widely used to estimate lesion size in 6-OHDA lesioned animals, with greater number of ipsilateral turns per min indicating a larger lesion. Following induction of motor activity with amphetamine treatment, sham animals performed both ipsilateral and contralateral turns in the rotometer. Therefore overall number of ipsilateral turns per min was small, with sham animals recording on average less than one turn per min (Figure 5.9). In contrast, unilateral 6-OHDA striatal lesions caused an imbalance of DA release following amphetamine treatment resulting in an ipsilateral turning response. Thus vehicle treated animals performed $9 \pm 2$ and $10 \pm 4$ ipsilateral turns per minute on days 7 and 14 post-lesion, respectively, which was a significant ipsilateral turning response compared to sham animals ($p < 0.01$). Chronic Captopril treatment further potentiated this ipsilateral turning response, with Captopril treated animals recording $14 \pm 1$ ipsilateral turns per min on both day 7 and 14 post-lesion, thus indicating a larger lesion. However Captopril treated animals did not display a significantly greater turning response than vehicle treated animals due to the large within group variation, which was particularly prominent in the vehicle treated group.
Figure 5.9: Chronic Captopril treatment – Estimation of lesion size as assessed by the rotometer.

Sham animals (black) did not demonstrate a marked ipsilateral turning response following induction of motor activity by the dopamine-releasing agent, amphetamine. Following 6-OHDA striatal lesions, vehicle treated animals (orange) performed only ipsilateral turns on both day 7 and 14 post-lesion and thus had a striatal dopamine deficiency or dopaminergic lesion. Captopril treatment (aqua) further potentiated this ipsilateral turning response, indicating that Captopril treated animals had a larger dopaminergic lesion than vehicle treated animals. (n=5/sham; n=6 / 6-OHDA groups).
5.3.2 Histological Outcome

Dopaminergic Response: TH Immunohistochemistry

The striatum of sham animals was clearly demarcated from the surrounding tissue, with a dense network of TH immunoreactive terminals throughout (Figures 5.10 and 5.11). 6-OHDA striatal lesions resulted in a progressive loss of striatal TH immunoreactive terminals. By day 3, vehicle treated animals had only a small loss of dopaminergic terminals, which increased by day 7 post-lesion, although the striatum remained well defined from surrounding tissue. Chronic Captopril treatment exacerbated the 6-OHDA induced loss of TH immunoreactive terminals. At day 3 post-lesion it was obvious that Captopril treated animals had a greater loss of striatal TH immunoreactive terminals than vehicle treated animals, although their striatum was still clearly demarcated at low magnification. This loss had increased by day 7 post-lesion, with the striatum of Captopril treated animals no longer being able to be distinguished from surrounding tissue. By day 21 post-lesion, 6-OHDA treated animals had a substantial loss of striatal dopaminergic terminals, although in Captopril treated animals very few TH immunoreactive terminals remained, as they were almost devoid of striatal TH immunoreactivity.

Despite the loss of DA terminals in the 6-OHDA groups at day 3 post-lesion, shams and both 6-OHDA groups demonstrated a normal distribution and number of dopamine neurons within their ipsilateral SN compared to their contralateral SN at day 3 post-lesion (Figures 5.12 and 5.13). This was expected as dopaminergic cell death is delayed in this model, with most neuronal death occurring from day 7 to 14 post-lesion (Lee et al., 1996). The loss of TH immunoreactive neurons was apparent by day 7 post-lesion, with vehicle animals recording an \(18 \pm 5\%\) cell loss in their ipsilateral SN, which was a
Figure 5.10: Chronic Captopril treatment – TH immunoreactivity within the ipsilateral striatum following 6-OHDA striatal lesions. TH stained sections (Bar = 90μm).

Intense TH immunoreactivity was observed throughout the striatum of sham animals (A). Following 6-OHDA striatal lesions, vehicle treated animals had a progressive loss of TH terminals that was not apparent until day 7 (D) post-lesion as day 3 (B) sections resembled shams. By day 21 (F) many of the TH immunoreactive terminals in vehicle treated animals had been lost. Captopril treatment exacerbated the loss of striatal TH immunoreactivity, with Captopril treated animals clearly having a greater loss of TH immunoreactive terminals than vehicles at day 7 (E) and 21 (G) post-lesion. However at day 3 (C) post-lesion, Captopril treated animals resembled vehicle treated and sham animals.
Figure 5.11: Chronic Captopril treatment – TH immunoreactivity within the ipsilateral striatum following 6-OHDA striatal lesions. TH stained sections (Bar = 50μm).

Sham animals (A) had intense TH immunoreactivity throughout the striatal section. Following 6-OHDA striatal lesions, vehicle treated animals had a progressive loss of TH immunoreactive terminals that was apparent by day 3 (B) post-lesion, and continued to increase throughout the assessment period with a greater loss of immunoreactivity on day 7 (D) then day 21 (F) post-lesion. Chronic Captopril treatment exacerbated this loss of TH immunoreactive terminals as Captopril treated animals had a profound loss of TH immunoreactivity. This loss was greater than observed in vehicle treated animals on days 3 (C), 7 (E), and 21 (G) post-lesion.
significant cell loss compared to sham animals ($p < 0.01$). By day 21, further
degeneration of dopaminergic neurons had occurred with only a few of the neurons
within the dorsolateral SN remaining. Vehicle treated animals recorded a $30 \pm 2\%$ loss of
TH immunoreactive neurons at this time. Captopril treatment further exacerbated this
degeneration of dopaminergic neurons on both day 7 and 21 post-lesion, with a $21 \pm 3\%$
and $48 \pm 5\%$ loss of TH immunoreactive neurons observed within the ipsilateral SN,
respectively (Figure 5.13), which was a significantly greater loss of nigral TH
immunoreactive neurons than vehicle treated animals at day 21 post-lesion ($p < 0.01$).
Thus chronic Captopril treatment accelerated dopaminergic degeneration induced by 6-
OHDA striatal lesions.

**General Pathology – H&E**

Sham animals had normal nigral architecture with many healthy neurons present in
different shapes and sizes, although an occasional dark cell was observed (Figure 5.14).
At day 3 following 6-OHDA striatal lesions, vehicle treated animals displayed similar
features to sham sections, with mainly healthy neurons present. However, by day 7 post-
lesion, these animals had a disruption in nigral architecture due to a loss of neurons.
Furthermore, the number of glial cells within the SN was increased over the 3-week
period. Captopril treated animals had a similar number of dopaminergic neurons to
vehicle treated animals on day 3 and 7 post-lesion, however many of the neurons were
highly stained with haematoxylin and therefore staining darker than other normal healthy
neurons. In H&E, neurons of this morphology are considered to be stressed or reversibly
injured. By day 21, no dark cells were present in either 6-OHDA group, although
infiltration of glial cells appeared to be increased in Captopril treated animals compared
to the vehicle treated animals.
**Figure 5.12:** Chronic Captopril treatment – TH immunoreactivity within the ipsilateral substantia nigra following 6-OHDA striatal lesions. TH stained sections (Bar = 200μm).

Sham animals (A) demonstrated normal numbers and distribution of TH immunoreactive neurons and fibers within the SN. Following 6-OHDA striatal lesions, no obvious loss of TH immunoreactive neurons was observed in vehicle treated animals until day 21 post-lesion (F), as at day 3 (B) and 7 post-lesion (D) distribution and number of TH immunoreactive neurons was normal in their ipsilateral SN. Captopril treated animals also demonstrated normal distribution of TH immunoreactive neurons at day 3 post-lesion (C). However, by day 7 post-lesion, Captopril treated animals (E) displayed the greatest loss of TH immunoreactive neurons and fibers within their ipsilateral SN of any group, with this loss further exacerbated on day 21 post-lesion (G).
**Figure 5.13:** Chronic Captopril treatment – Quantification of TH immunoreactive neurons within the ipsilateral substantia nigra.

Sham animals (black) displayed no loss of TH immunoreactive neurons within their ipsilateral SN during the assessment period. Following 6-OHDA striatal lesions, vehicle treated animals (orange) demonstrated degeneration of dopaminergic or TH immunoreactive neurons by day 7 post-lesion, with a subsequent increase in cell death by day 21 post-lesion. Captopril treatment exacerbated this loss of TH immunoreactive neurons, with Captopril treated animals (aqua) recording an increased percentage of cell loss to vehicle treated animals on day 7 and 21 post-lesion. At this later time, TH cell loss was significantly greater. (** denotes p < 0.01 versus vehicle) (n=5/sham; n=6 / 6-OHDA groups).
Figure 5.14: Chronic Captopril treatment - H&E stained ipsilateral substantia nigra following 6-OHDA striatal lesions. H&E stained sections (Bar = 50μm).

Sham animals (A) had normal nigral architecture, with many healthy neurons present. No obvious change in nigral architecture or cellular integrity was seen at day 3 post-lesion in either 6-OHDA groups (B; vehicle), although neurons in the Captopril group (C) were slightly darker in colour. By day 7, vehicle (D) and Captopril (E) treated animals demonstrated a loss of neurons and nigral architecture, with occasional neurons within the Captopril treated animals showing features of dark cell change. Subsequently, by day 21 both 6-OHDA groups had abnormal nigral architecture, however infiltration of glial cells was greater in the Captopril treated animals (G) than vehicle treated animals (F).
**Substance P Response – SP Immunohistochemistry**

Sham animals displayed basal levels of SP immunoreactivity within their striatum and SN, with the intensity of the immunoreactivity greater in the latter region due to the higher expression of SP found there (Figures 5.15 and 5.16). Within the striatum (Figure 5.15), SP immunoreactivity was observed in perivascular tissue and in both medium spiny projection neurons and large interneurons. Following 6-OHDA striatal lesions, vehicle treated animals demonstrated an increase in SP immunoreactivity throughout the striatum, although no obvious change in neuronal SP production was observed. This increase persisted until day 21 post-lesion, however was greatest on days 3 and 7 post-lesion. Captopril treated animals also displayed increased SP immunoreactivity in their striatum relative to shams at each day post-lesion, and also had a slight increase in striatal immunoreactivity compared to vehicles on days 3 and 7 post-lesion, particularly in perivascular tissue. This increase may have been due to reduced degradation of SP by inhibition of ACE. Nonetheless by day 21 post-lesion, Captopril treated animals had comparable SP immunoreactivity to vehicle treated animals.

In the SN (Figure 5.16), sham sections had intense SP immunoreactivity within nigral tissue, and although intensity of immunoreactivity varies between neurons, there was faint SP immunoreactivity within nigral neurons. At day 3 and 7 post-lesion, vehicle treated animals had similar immunoreactivity to shams, with faint immunoreactivity within most neurons. In contrast, Captopril treated animals had a few neurons with intense cytoplasmic SP immunoreactivity. This increased immunoreactivity was still apparent at day 21 post-lesion, although at this time many neurons in both 6-OHDA groups display increased cytoplasmic SP immunoreactivity. No loss of nigral SP immunoreactivity was observed in any groups at any day post-lesion.
Figure 5.15: Chronic Captopril treatment - SP immunoreactivity within the ipsilateral striatum following 6-OHDA striatal lesions. SP stained sections (Bar = 25μm).

Sham animals (A) demonstrated normal levels of SP immunoreactivity within the ipsilateral striatum and in perivascular tissue. Following 6-OHDA striatal lesions, vehicle treated animals displayed increased immunoreactivity within the striatum by day 3 (B), with SP immunoreactivity still slightly elevated at day 7 (D) and 21 (F) post-lesion. Captopril treated animals also had greater SP immunoreactivity than shams, but also had increased SP, particularly perivascularly, compared to vehicle treated animals on days 3 (C) and 7 (E) post-lesion. However, by day 21 post-lesion, SP immunoreactivity in Captopril treated animals (G) was comparable to vehicle treated animals.
Figure 5.16: Chronic Captopril treatment - SP immunoreactivity within the ipsilateral substantia nigra following 6-OHDA striatal lesions. SP stained sections (Bar = 25μm).

Sham animals displayed intense SP immunoreactivity throughout nigral tissue, with faint cytoplasmic immunoreactivity within most neurons. Following 6-OHDA striatal lesions, no obvious change in SP immunoreactivity was observed in vehicle treated animals at either day 3 (B), 7 (D) or 21 (F) post-lesion, although by day 7 and 21 a few neurons had intense cytoplasmic immunoreactivity. However, in Captopril treated animals this increase in neuronal SP immunoreactivity was evident by day 3 post-lesion (C) and persisted on days 7 (E) and 21 (G) post-lesion.
Astrocytic Response – GFAP Immunohistochemistry

Sham animals displayed normal GFAP expression within the striatum, with immunoreactivity around blood vessels, white matter and in circulating resting astrocytes (Figure 5.17). Similar GFAP immunoreactivity was observed within the SN of sham animals (Figure 5.18). Within the striatum, vehicle treated animals demonstrated a profound astrocytic response following 6-OHDA striatal lesions, with an increase in GFAP immunoreactivity throughout the striatum, and upregulation of GFAP within hypertrophic astrocytes as early day 3 post-lesion. Although by day 7, the striatal expression of GFAP had subsided, the number of astrocytes and the intensity of GFAP expression within them had not reduced. Thus these astrocytes remained in an activated state. By day 21 post-lesion, numerous astrocytes were still present, although GFAP immunoreactivity was less intense within many of the remaining astrocytes. Captopril treated animals displayed a similar astrocytic response following 6-OHDA striatal lesions, although GFAP immunoreactivity was greater than in vehicle treated animals. Accordingly, Captopril treated animals had a massive upregulation of GFAP expression within the entire striatal section as early as day 3 post-lesion, which persisted to a lesser degree at days 7 and 21. Furthermore, throughout the entire assessment period, increased numbers of activated astrocytes, which were hypertrophic and displayed intense GFAP immunoreactivity, were observed within Captopril treated animals.

A similar increase in GFAP immunoreactivity was observed within the SN following 6-OHDA striatal lesions, however in vehicle treated animals the increase was delayed, with a re-distribution from white matter to around dopaminergic neurons occurring before an increase in GFAP immunoreactivity and number of activated astrocytes. Maximal GFAP
Sham animals (A) demonstrated normal intensity and distribution of GFAP immunoreactivity within their striatum. Following 6-OHDA striatal lesions, vehicle treated animals had an upregulation in striatal GFAP immunoreactivity by day 3 post-lesion (B), with an increase in the number and size of astrocytes. These astrocytes are displaying greater GFAP immunoreactivity than astrocytes in shams. Although by day 7 vehicle treated animals had slightly decreased GFAP immunoreactivity, a high number of hypertrophic neurons remained at day 7 (D) and 21 (F) post-lesion. An exacerbated astrocytic response was observed in Captopril treated animals, with greater GFAP immunoreactivity and number of hypertrophic astrocytes than vehicle treated animals seen on day 3 (C), 7 (E) and 21 (G) post-lesion.

**Figure 5.17: Chronic Captopril treatment - GFAP immunoreactivity within the ipsilateral striatum following 6-OHDA striatal lesions.** GFAP stained sections (Bar = 50μm).
Figure 5.18: Chronic Captopril treatment - GFAP immunoreactivity within the ipsilateral substantia nigra following 6-OHDA striatal lesions. GFAP stained sections (Bar = 50\(\mu\)m).

Sham animals (A) displayed normal intensity and distribution of GFAP immunoreactivity within their SN. Following 6-OHDA lesions, vehicle treated animals had a redistribution of GFAP immunoreactivity from white matter to around dopaminergic neurons at day 3 (B) before GFAP immunoreactivity was increased at day 7 (D) post-lesion, with a greater number of intense GFAP immunoreactive astrocytes present. Maximal GFAP immunoreactivity was observed in vehicle treated animals on day 21 (F). In contrast, Captopril treated animals demonstrated increased GFAP immunoreactivity by day 3 (C) post-lesion, with maximal GFAP immunoreactivity seen by day 7 (E). Many hypertrophic astrocytes with intense GFAP immunoreactivity were present at this time, and were still observed at day 21 (G) post-lesion.
immunoreactivity was seen in vehicle treated animals on day 21, whereas Captopril treated animals had maximal GFAP expression on day 7. The increase in GFAP immunoreactivity in Captopril treated animals was observed by day 3 post-lesion, when immunoreactivity was surrounding dopaminergic neurons. By day 21 post-lesion, Captopril animals showed decreased immunoreactivity compared to vehicles, although the number of activated astrocytes still remained high.

**Activated Microglial Response – ED-1 Immunohistochemistry**

ED-1 immunoreactivity is a marker of the complement protein CD-68, which is expressed by phagocytic macrophages and microglia in the brain. Accordingly, ED-1 immunoreactivity is a measure of brain microglial activation. As expected, sham animals displayed no apparent ED-1 immunoreactivity within the striatum (Figure 5.19), and only an occasional perivascular cell within the SN (Figure 5.20). However, activation of microglia or an increase in ED-1 immunoreactivity was observed following 6-OHDA striatal lesions. Within the striatum, only a few activated microglia were present by day 3 post-lesion in vehicle treated animals. In these animals, ED-1 immunoreactivity progressively increased over the 3-week period so that maximal expression was seen on day 21 post-lesion. A similar progressive increase in ED-1 immunoreactivity was observed in Captopril treated animals, but compared to vehicle treated animals, the number of activated microglia was substantially greater, particularly at day 21 post-lesion. A progressive increase in ED-1 immunoreactivity was also seen in the SN, yet the increase was delayed, with both vehicle and Captopril treated animals resembling sham animals at day 3 post-lesion. However, by day 7 post-lesion, activated microglia are present within the SN, mainly near dopaminergic neurons in both vehicle and Captopril treated animals. Maximal ED-1 immunoreactivity for both 6-OHDA groups was observed
Figure 5.19: Chronic Captopril treatment – ED-1 immunoreactivity within the ipsilateral striatum following 6-OHDA striatal lesions. ED-1 stained sections (Bar = 50\(\mu\)m).

Sham animals (A) displayed no ED-1 immunoreactivity and therefore presence of activated microglia within their striatum. In contrast, following 6-OHDA striatal lesions, ED-1 immunoreactivity was observed in vehicle treated animals by day 3 (B), with a progressive increase in ED-1 immunoreactivity or number of activated microglia on days 7 (D) and 21 (F) post-lesion. A similar pattern of ED-1 immunoreactivity was seen in Captopril treated animals, with a progressive increase in activated microglia on days 3 (C), 7 (E) and 21 (G) post-lesion. However, at all times post-lesion, greater ED-1 immunoreactivity was observed in Captopril treated animals than vehicle treated animals.
Figure 5.20: Chronic Captopril treatment – ED-1 immunoreactivity within the ipsilateral substantia nigra following 6-OHDA striatal lesions. ED-1 stained sections (Bar = 50μm).

Sham sections (A) displayed only an occasional perivascular glial cell with ED-1 immunoreactivity. Following 6-OHDA striatal lesions, ED-1 immunoreactivity and therefore activation of microglial cells, was not apparent until day 7 post-lesion in either the vehicle (D) or Captopril (E) treated animals, as day 3 sections (B and C, vehicle and Captopril, respectively) resembled shams. Maximal ED-1 immunoreactivity was observed at day 21 in both the vehicle (F) and Captopril (G) treated animals, however, Captopril treated animals had greater ED-1 immunoreactivity than vehicle treated animals.
observed at day 21 post-lesion, although as in the striatum, a greater number of activated microglia were seen in Captopril treated animals on both days 7 and 21 post-lesion.

**Blood Brain Barrier Dysfunction – Albumin Immunohistochemistry**

Albumin immunoreactivity is often used as a marker of BBB dysfunction, with increased immunoreactivity indicative of barrier breakdown. As expected, sham animals did not display albumin immunoreactivity within the brain section itself, only around the perimeter and in the ventricles and blood vessels where the BBB is located (Figure 5.21). Following 6-OHDA striatal lesions, albumin immunoreactivity increased within the vehicle animals ipsilateral striatum and subcortex at day 3 post-lesion, indicating BBB breakdown had occurred. By day 7, albumin immunoreactivity had largely disappeared, with immunoreactivity only present along the edge of the ipsilateral striatum. Captopril treated animals also demonstrated a breakdown in the BBB on day 3 post-lesion, although albumin immunoreactivity was greater than in vehicle treated animals, being visible across the entire ipsilateral hemisphere and into the contralateral cortex. Furthermore, albumin immunoreactivity was still present on day 7 post-lesion within the dorsolateral striatum. Thus chronic Captopril treatment with 6-OHDA striatal lesions resulted in substantial BBB dysfunction during the first week following lesioning.
Figure 5.21: Chronic Captopril treatment – Albumin immunoreactivity following 6-OHDA striatal lesions. Albumin stained sections.

Sham sections (A) displayed faint albumin staining around the surface of the brain, and within ventricles and blood vessels. Following 6-OHDA striatal lesions, vehicle treated animals demonstrated an increase in albumin immunoreactivity by day 3 (B) post-lesion, with staining seen in the ipsilateral cortex and striatum. By day 7, albumin immunoreactivity had decreased in vehicle treated animals (D), although was still evident within the ipsilateral corpus callosum. Chronic Captopril treatment further increased albumin immunoreactivity on days 3 (C) and 7 (E) post-lesion, with the entire ipsilateral hemisphere immunoreactive for albumin by day 3 and the dorsolateral striatum immunoreactive for albumin by day 7.
5.4 Discussion

The present study demonstrated that chronic treatment with the ACE inhibitor, Captopril, in an early stage rat model of PD accelerates injury by increasing dopaminergic terminal loss and subsequent cell death as well as exacerbating functional deficits. This dopaminergic degeneration and poor functional outcome in Captopril treated animals, particularly motor function in the first week, was associated with increased SP immunoreactivity, specifically perivascular SP, a greater inflammatory response, and a substantial breakdown in the BBB.

ACE inhibitors are widely used for the treatment of hypertension and chronic heart failure. The main target of ACE is angiotensin II, which as well as being central to the renin-angiotensin system, is found in the striatum of rats with its receptor, Angiotensin II type 1 (AT1), and in humans where it is associated with the nigro-striatal dopaminergic system (Jenkins et al., 1997). Here AT1 is located both on DA neurons and their terminals. Additionally, ACE is involved in the metabolism of SP, high concentrations of which are normally found within the SN, GP and striatum. Consequently ACE activity within the basal ganglia is also high in rats (Jenkins et al., 1997) and humans (Arinami et al., 1996). In the cerebrospinal fluid of PD patients, the concentration of the AT1 receptor is decreased, and that of ACE elevated (Rodriguez-Pallares et al., 2004).

Previous studies have reported a beneficial effect of chronic treatment with ACE inhibitors in experimental models of PD where massive dopaminergic cell death occurs within hours, thus reproducing the late stages of the disease, as well as in moderate to severe human PD (Kurosaki et al., 2005; Lopez-Real et al., 2005; Munoz et al., 2006; Reardon et al., 2000). ACE inhibitors have been shown to possess antioxidant properties
due to their ability to inhibit the formation of striatal angiotensin II, which utilizes ROS in its signalling (Lopez-Real et al., 2005). Furthermore, angiotensin II is able to activate NADPH oxidase, which is the main source of intracellular ROS, apart from mitochondria (Munoz et al., 2006). Dopaminergic neurons are normally in a state of oxidative stress because of both the enzymatic catabolism by MAO and the autooxidation of DA itself, which produce of H₂O₂ and ROS (Olanow et al., 2004). In experimental models of PD, increased formation of these free radicals results in lipid peroxidation and protein oxidation, leading to dopaminergic cell death. Thus, Captopril treatment has been reported to decrease in oxidative damage and preserve dopamine neurones in both 6-OHDA and MPTP models that replicate late PD (Lopez-Real et al., 2005; Munoz et al., 2006). Furthermore, chronic Captopril treatment increases striatal DA content and release (van den Buuse et al., 2005). This effect is likely to be caused by the ability of ACE inhibitors to modulate SP levels, which can cause the release of DA (Jenkins et al., 1999; Kurosaki et al., 2005). The release of SP and therefore DA is further potentiated by increased bradykinin, which promotes additional synthesis and release of SP and is also a substrate for ACE (Jenkins et al., 1997). Such a scenario would be advantageous in the late stages of PD, when SP expression is decreased and DA release is inadequate.

Despite the aforementioned positive effects of treatment with ACE inhibitors in PD, they have only been tested in models replicating the late stages of the disease and their effects have not been studied in the early stages. Accordingly, the current study examined chronic Captopril treatment in an intrastriatal 6-OHDA model of PD, which replicates the early stages of the disease. Unlike later in the disease, Captopril treatment was detrimental to motor function, especially within the first week in early PD, with Captopril treated animals exhibiting significant akinesia, bradykinesia and gait abnormalities when
compared to vehicle treated animals. This is likely due to the exacerbated striatal DA terminal loss at day 3 post-lesion in Captopril treated animals, which would result in insufficient striatal DA and therefore abnormal function of the basal ganglia. However, these animals showed improvement in motor function on the rotarod and stepping tests by day 7, which could be due to the instigation of compensatory mechanisms such as increased DA synthesis and release, which may be a potentiated by greater availability of SP, as well as upregulation and increased sensitivity of the DA receptors (Deumens et al., 2002; Perez-Otano et al., 1998).

Despite the improvement, Captopril treated animals consistently performed below vehicle treated animals, as their exacerbated dopaminergic terminal loss in the striatum produced a profound reduction in striatal dopamine that may not have been fully prevented by the additional SP potentiated DA release. Furthermore, this enhanced synthesis of DA would have imposed high levels of oxidative stress within the SN and may have exacerbated dopaminergic cell death seen at day 21 in Captopril treated animals. Indeed, in Captopril treated animals, many of their dopaminergic neurons, particularly early following 6-OHDA striatal lesions, appeared dark on H&E assessment. This type of cellular staining suggests these neurons are stressed or reversibly injured, and therefore it may be these neurons that are most vulnerable and likely to degenerate. Due to the exacerbated loss of striatal dopaminergic terminals early, Captopril treated animals dopaminergic neurons were under greater stress than vehicle treated animals.

Whilst no evidence exists to date about the expression of SP early in human PD when dopaminergic cell death is occurring, it is known that compensatory mechanisms are in effect. One could therefore speculate that as SP plays a role in DA release, that its
expression could also be elevated to help with the extra demand. Indeed, in the current study, SP was increased in both the striatum early following 6-OHDA striatal lesions, and within nigral neurons at later times. Moreover, in other experimental models of PD, an increase in extracellular SP within the SN (Orosz and Bennett, 1990) and in GP neurons has been observed (Martorana et al., 2003). However, high levels of SP have been associated with cell death and functional deficits in other brain pathologies (Donkin et al., 2007; Turner et al., 2007).

While Captopril treated animals had an exacerbated loss of dopaminergic terminals and neurons and profound motor and neurological deficits, the open field test, which assessed stress and anxiety of the animal through spontaneous exploration, demonstrated that Captopril treated animals recorded higher activity levels than sham or vehicle treated animals, except for day 3 post-lesion. At this time, increased SP levels, which has been attributed to stress and anxiety, was observed in both vehicle and Captopril treated animals. Both vehicle and Captopril treated animals took longer to become habituated to the task than shams, which may have been due to a loss of dopaminergic neurons within the VTA, an event that is exacerbated in Captopril animals. The VTA projects axons to the amygdala, medial prefrontal cortex and nucleus accumbens. These brain regions are important regulators of fear and anxiety and a loss of dopaminergic input to these areas will attenuate these emotions (Pezze and Feldon, 2004). Moreover, lesions of the amygdala result in decreased habituation to the open field task (Daenen et al, 2001). Dopaminergic projections from the VTA to the hippocampus are also present in the rat, suggesting a role for DA in hippocampal-dependent memory (Gasbarri et al., 1997). Many studies have reported learning and memory deficits following 6-OHDA lesions
(Ferro et al., 2005), and these deficits may contribute to the delay in habituation of 6-OHDA animals seen in this study in the open field task.

SP is co-localised with classical neurotransmitters such as glutamate and GABA, highly important regulatory neurotransmitters involved in basal ganglia function. Specifically, striatal glutamate release requires the activation of NK₁ receptors by SP (Marti et al., 2005). Therefore, elevated SP levels may potentiate increased striatal glutamate release. Excess glutamate results in elevated intracellular Ca²⁺, which produces free radicals, mitochondrial damage and activation of proteases, endonucleases and phospholipases. This deleterious series of events is known as glutamate excitotoxicity, which has been previously mentioned is a major contributor to cell death in PD (Beal, 1992). Methamphetamine treatment will cause dopaminergic cell death due to a substantial increase in both DA and glutamate, resulting in oxidative stress and glutamate excitotoxicity (Golembiowska et al., 2002). SP and the striatal NK₁ receptors have been shown to mediate methamphetamine toxicity and cell death as pre-treatment with an NK₁ antagonist was protective (Loonam et al., 2003). Furthermore, an increase in glutamate lead to increased NO levels, which can also be increased by SP through its ability to induce NOS (Kang et al., 2001), which is present in microglia, astrocytes and large striatal interneurons. NO contributes to oxidative stress and subsequent dopaminergic cell death in PD by producing the highly reactive OH⁻ radical and ONOO⁻, both powerful oxidising agents (Guix et al., 2005; Olanow and Tatton, 1999; Beal, 1998). Additionally, when SP binds to NK₁ receptors, which are present on striatal and DA neurons, an increased turnover of intracellular IP₃ occurs with a subsequent rise in intracellular Ca²⁺ that triggers additional SP release, creating a positive feedback cycle (Gerard et al., 1991; Saria, 1999). Elevated levels of IP₃ and Ca²⁺ may also activate phospholipase A₂ (PLA₂)
resulting in the release of arachidonic acid (AA), the metabolism of which can potentiate inflammation (Mitchell and Cotran, 1997).

Inflammation is a key contributor to cell death in PD. Previous studies have shown an increase of up to 1500% in the inflammatory mediators interleukin-1β (IL-1β) and TNF-α in the SN, striatum and CSF of PD patients (Jenner and Olanow, 1998). Within the SN, high numbers of microglia reside, which along with its increased state of oxidative stress, makes the SN particularly sensitive to insults, especially in PD where glutathione, the principle antioxidant defence of mitochondria, is reduced (Teismann and Sculz, 2004). Indeed, dopaminergic degeneration by intrastriatal injections of 6-OHDA has previously been associated with activation of microglia both initially after the lesion and one week later as dopaminergic neurons begin to die (He et al., 2001). As was seen in the current study, this microglial response can remain for weeks after the lesion (Cicchetti, et al. 2002). Notably SP has been shown to activate microglia and promote their chemotaxis (Block et al., 2006; Rasley et al., 2002). Once activated, microglia produce pro-inflammatory factors and cytokines including IL-1β, interleukin-2 and -6, and TNF-α, as well as NO, ROS and AA metabolites, all of which are cytotoxic to dopaminergic neurons (Arai et al., 2006; Hirsch, 2000). Accordingly, previous studies have shown that microglia are essential in the inflammatory response that occurs following stroke or brain trauma and in both acute and chronic neurodegeneration (Perry, 2004). In our study, the increased availability of SP in Captopril treated animals may have resulted in greater activation of microglia and therefore potentiated the accelerated dopaminergic cell death observed in these animals. Furthermore, SP is able to activate NADPH oxidase located in microglia (Serra et al., 1988), and NADPH oxidase is known to produce high levels of
intracellular superoxide free radicals, which along with other ROS have been shown to play a major role in the degeneration of DA neurons (Gao et al., 2002).

Astrocytes are also known to express NK1 receptors and therefore are activated by SP. Following a 6-OHDA striatal lesion, an astrocytic response was initiated, however, in Captopril treated animals this activation of astrocytes was greater than in vehicle treated animals, supporting the role of SP in activation of astrocytes. Astrocytes are thought to be both neuroprotective due to their ability to secrete neurotrophic factors such as GDNF, and neurotoxic by secreting proinflammatory cytokines. It is unknown exactly what role they are playing in the current study, whether they are upregulated more in Captopril treated animals to protect the many degenerating neurons or whether they are contributing to the death of the neurons. Nonetheless, inflammation is important in cell death in chronic neurodegenerative disorders, as well as exacerbation of functional deficits. For example in Alzheimer’s disease, inflammatory processes are deleterious to outcome as patients who developed a systemic infection with raised serum IL-1β levels had a greater cognitive decline over a 2 month period following the infection than those patients who had not had an infection (Holmes et al., 2003). Moreover, in the current study, the greatest functional deficits were observed in the Captopril treated animals, which displayed the most extensive inflammatory response.

Recently, BBB breakdown, which would allow an influx of inflammatory cells, has been shown to occur in PD and is thought to accelerate the progression of the disease (Kortekaas et al., 2005). In trauma and stroke, increased SP release has been shown to be associated with barrier breakdown due to instigation of neurogenic inflammation by SP. Confirmation of the role of SP in BBB dysfunction was observed in the current study,
with increased striatal SP immunoreactivity occurring concurrently with barrier breakdown, which was further potentiated in Captopril treated animals where SP degradation was reduced. Moreover, these animals had accelerated disease progression compared to vehicle treated animals. Thus neurogenic inflammation, which results in plasma extravasation, vasodilation and BBB dysfunction, may play an important role in dopaminergic degeneration in PD.

5.5 Conclusions

Chronic treatment with ACE inhibitors accelerated injury in the 6-OHDA intrastriatal model of early PD replicating delayed and progressive dopaminergic cell death. In particular, motor deficits such as bradykinesia, akinesia and gait abnormalities that define human PD, were more pronounced in Captopril treated animals due to the greater loss of striatal DA terminals and exacerbated loss of ipsilateral dopaminergic neurons. Furthermore, increased SP expression observed in Captopril treated animals resulted in greater activation of microglia and astrocytes within the striatum and SN. Similarly there was greater BBB breakdown. Taken together, all of these effects are likely to contribute to the increased loss of dopaminergic terminals and neurons. Although in the later stages of PD, Captopril has been shown to be beneficial, these conflicting results can be explained by the different amounts of SP being produced during the progression of the disease. Limiting its degradation may be favourable in the late stages of the disease when SP levels are decreased but not in the early stages of the disease. As such, the timing of ACE inhibitor treatment for PD may be critical depending on the amount of SP available to dopaminergic cells throughout the progression of the disease.