CONFIDENTIAL

Effects of COMPOUND XXX in an Animal Model of Parkinson's Disease

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2 Authentication

'I, the undersigned, hereby declare that this work was performed under my direction and the study was conducted according to the procedures herein described and this report represents a true and accurate record of the results obtained. I, the undersigned declare, that the following report constitutes a true and faithful account of the procedures adopted, and the results obtained in the performance of this study. This study was formally exempt from compliance of Good Laboratory Practice regulations. However, the principles and procedures of the Good Laboratory Practice were followed to the maximal possible extend.

_____

Date

Study Author

Hitherto, we accept this final report in its present form.

_____

Study Co-ordinator

_____

Director

3 Personnel Involved

Study Director:

Project Performed by:

Responsible for animal care

4 Summary

COMPOUND XXX was tested for the purpose of determining whether the drug would influence the L-DOPA induced contralateral rotations in a rodent model of Parkinson's disease where there is a nearly complete lesion of the dopamine nigrostriatal pathway. The animals were given the nigrostriatal lesion first and then 1 month later COMPOUND XXX was administered 3 hours prior to the injection of either a low (3.125 mg/kg) or high (25 mg/kg) dose of L-DOPA. The number of rotations was then counted for the next 3 hours in 30 minute bins. The administration of COMPOUND XXX and L-DOPA continued for either 22 or 23 days.

Male Sprague-Dawley rats were randomised into 7 treatment groups, each containing either 9 or 10 animals.

The following groups were tested:

Group 1: low L-DOPA (3.125 mg/kg) + vehicle (1% HPMC)
Group 2: low L-DOPA dose (3.125 mg/kg) + low COMPOUND XXX(25 mg/kg)
Group 3: low L-DOPA (3.125 mg/kg) + high COMPOUND XXX(75 mg/kg)
Group 4: high L-DOPA (25 mg/kg) + vehicle (1% HPMC)
Group 5: high L-DOPA dose (25 mg/kg) + low COMPOUND XXX(25 mg/kg)
Group 6: high L-DOPA (25 mg/kg) + high COMPOUND XXX(75 mg/kg)
Group 7: acute high L-DOPA dose (25 mg/kg) only on day 23.

Of the initial 70 animals that were lesioned with the neurotoxin, 6-OHDA, 4 animals died. Upon administration of the high dose of L-DOPA, several animals died in those groups (groups 5 and 6).

Results indicate that
1.) overall mean rotation rates for the 3 hours of testing of the high dose L-DOPA over the 5 test days did not differ from each other (see tab: Group mean rotation for data and Figure 1). However, when the individual groups were analyzed separately, there was a difference in Groups 4, 5 and 6 at the various testing days. For Groups 4, 5, and 6, there was a difference in the mean number of rotations for day 1 compared to all the other days ($p < .005$). For only Group 6, there was also a difference between Day 8 and Day 15 ($p = .045$).
2.) the low L-DOPA treated groups showed very few rotations and administration of COMPOUND XXX at either dose showed little effect on rotation numbers (see tab: Group mean rotation for data).
3.) when the individual days are analyzed over the 3 hour time period of testing, some differences are seen at the earlier test days versus the later test days (see tab: Mean rotations by date). Considering that the low L-DOPA groups showed few rotations compared to the high L-DOPA rotations, the data for the low and high L-DOPA groups were analyzed separately (see tab: mean rot hi LD vs lo LD). For test days 1 and 8, there was a statistical difference between the low and high doses of the COMPOUND XXX+ high L-

DOPA groups (ie groups 5 and 6) compared to the vehicle + high L-dopa (group 4) at either the 30 or 60 minute time point, respectively. There was no difference between the two COMPOUND XXX+ high L-DOPA groups (i.e., no difference between groups 5 and 6). By test day 15, 22 or 23, there was no difference between any of the high L-DOPA groups.

4.) There was no difference in the times for the animals to peak in terms of rotations when all groups were compared to each other (Figure 2) (see tab; Group Peak Time). However, when the individual groups were analyzed separately, there was a difference in peak times for only Group 5. The differences in peak times occurred only between Days 8 and 22.

5.) All animal groups had approximately the same degree of loss of TH immunolabeling on the lesioned side (85-90%).

5 Introduction

The purpose of the study was to determine the effects of administration of COMPOUND XXX on L-DOPA induced contralateral rotations over a 3 week time period. Two different doses of COMPOUND XXX were administered, along with two different doses of L-DOPA. For L-DOPA, a low dose was chosen so that few, if any, of the rats would show rotations. COMPOUND XXX was first administered, then 3 hours later, L-DOPA. In this manner, it would be determined if COMPOUND XXX would cause more rotations to occur compared to L-DOPA alone. For the high-dose L-DOPA study, a sufficiently high dose was chosen to assure that all rats would rotate the first time the drug was given. In this manner, it could be determined if COMPOUND XXX would decrease the number of L-DOPA induced contralateral rotations. There was also the possibility that at such a high dose of L-DOPA, COMPOUND XXX could potentially augment the number of rotations.

6 Experimental Procedure

6.1 Test Item

Compound XXX, benserazide (provided by Company XXX), L-DOPA (purchased from Sigma).

Compound XXX: used as a suspension in 1% HPMC and was made up fresh every 2 days. The suspension was stored in the refrigerator when not in use. Several vials of only the suspension gel were made ahead of time and kept in the refrigerator. Due to the large number of animals and the frequency of administration of the Compound XXX, the formulation was made fresh every other day.

Benserazide was used at a dose of 15 mg/kg. Drug was dissolved in the L-DOPA solution. The solvent contained normal saline (0.9% sodium chloride) and 1% ascorbic acid, to block the oxidation of L-DOPA. Fresh drug was made up every other day. There was no indication of oxidation/discoloration of this solution.

L-DOPA was used at either 3.125 mg/kg or 25 mg/kg. The drug was dissolved in normal saline with 1% ascorbic acid and injected IP in a volume of either 0.2 ml (low dose) or 2.5 ml/rat (high dose). The reason for the difference in volume injected was that L-DOPA purchased from Sigma was the base and not the salt. The base has a limited solubility and therefore a larger volume of the drug needed to be injected for the high dose L-DOPA group. The L-DOPA base was used in our previously published studies and we did not want to change to a different formulation of the drug. Fresh drug was made up every other day. There was no indication of oxidation/discoloration of this solution.

6.2 Location of Study

Bldg #, Room #

6.3 Animals and Management

6.3.1 Animals

Rat, Sprague-Dawley, males, initial weight prior to lesion was about 280-300 grams. 70 rats were ordered and all rats were lesioned using a unilateral injection of 6-OHDA into the medial forebrain bundle. Initial success of the lesion was tested by administration of amphetamine (5 mg/kg, ip), and the number of ipsilateral rotations counted for 5 minutes starting 15 minutes after the injection. All animals rotated greater than 5 times/minute. A final evaluation of the success of the lesion was determined by immunolabeling of the substantia nigra pars compacta (SN-PC) using an antibody against tyrosine hydroxylase (TH), the late limiting enzyme used in the synthesis of dopamine. The relative optical density of TH labeling in the SN-PC on the

lesioned vs unlesioned side were compared (see separate file, Company XXX Optical Density). With a complete lesion of the nigrostriatal pathway, there are few, if any TH labelled cells left on the lesioned side. Therefore, the relative optical density of the lesioned vs unlesioned side was evaluated and the percent decrease in optical density between the lesioned and non-lesioned sides was determined.

6.3.2 Acclimatisation and housing conditions

The animals were allowed to acclimatise for 7 days before the study started.

There was automatic control of light cycle, temperature and humidity. Light hours were 0600-1800h. Daily monitoring indicated that temperature and humidity remained within the target ranges of 20°C ± 2°C and 50% ± 2% respectively.

The animals were housed in polypropylene cages (30.80cm x 30.80cm x 18.72cm), with mesh tops from arrival x 2 rats per cage. Cages, bedding, and water bottles were changed at regular intervals, i.e., every 3 days.

Standard Diet was available to the animals ad libitum. The animals had access to domestic quality mains water *ad libitum*.

To provide environmental enrichment, no other items were made available to the animals.

6.4 Surgery

The rats were anesthetized with isoflurane, injected with desipramine (10 mg/kg, i.p.) and pargyline (40 mg/kg, i.p.), placed in the stereotaxic apparatus and the left medial forebrain bundle injected with 1 ul of 6-OHDA (2 ug/ul in 1% ascorbic acid) over a 1 minute period at two different sites: rostral-caudal from Bregma: -4.3 and -4.8 mm; lateral: 1.6 mm; dorsal-ventral: 8.8 mm. The needle was left in place for 5 minutes before withdrawing. The animals were left to recover for 4 weeks prior to drug administration.

6.5 Treatment

6.5.1 Treatment Groups

On arrival all animals were randomly allocated to treatment groups, such that the treatment groups were evenly distributed throughout the caging system.

The treatment groups and animal numbers were arranged as follows:

SUMMARY

	Day 0	Day 1	Day 8	Day 15	Day 22	Day 23	Day 24
Group 1	Veh + veh	LD lo + veh	LD lo + veh	LD lo + veh	LD lo + veh	not tested	brain
Group 2	veh + XXX lo	LD lo + XXX lo	LD lo + XXX lo	LD lo + XXX lo	LD lo + XXX lo	not tested	brain
Group 3	veh + XXX hi	LD lo + XXX hi	LD lo + XXX hi	LD lo + XXX hi	LD lo + XXX hi	not tested	brain
Group 4	veh + veh	LD hi + veh	LD hi + veh	LD hi + veh	LD hi + veh	LD hi + veh	brain
Group 5	veh + XXX lo	LD hi + XXX lo	LD hi + XXX lo	LD hi + XXX lo	LD hi + XXX lo	LD hi + XXX lo	brain
Group 6	veh + XXX hi	LD hi + XXX hi	LD hi + XXX hi	LD hi + XXX hi	LD hi + XXX hi	LD hi + XXX hi	brain
Group 7	not tested	not tested	not tested	not tested	not tested	LD hi + veh	brain

There is no non-lesioned control group since the purpose of the study was to determine if COMPOUND XXX had any affect on L-DOPA-induced rotations. In unlesioned animals, L-DOPA administration does not result in any rotations. All animals received a unilateral 6-OHDA lesion. Four rats unexpectedly died 2-3 weeks after the lesion, leaving a total of 66 rats. Group 1 had 9 rats, groups 2 through 6 had 10 rats each, and group 7 had 7 rats. During the course of the study, 4 rats died from group 6 and 1 rat died from group 5. Doses of COMPOUND XXX were either 25 mg/kg x 2/day (XXX lo) or 75 mg/kg x 2/day (XXX hi). For L-DOPA, the doses were either 3.125 mg/kg x 2/day (LD lo) or 25 mg/kg x 2/day (LD hi).

6.5.2 Selection of Dose Levels

Dose levels of COMPOUND XXX were agreed with the Sponsor following evaluation of existing relevant data. A high dose of 75 mg/kg x 2/day or a low dose of 25 mg/kg x 2/day was used as recommended by the Sponsor. Dose levels took into account the anticipated therapeutic dose i.e. leading to plasma levels close to the affinity at the target.

6.5.3 Route and Means of Administration

The animals were dosed with COMPOUND XXX orally by gavage at a volume of 2.5 ml dosing solution per animal using a steel dosing cannula. L-DOPA + benserazide (mixed together) was given IP in normal saline. The volume administered to each animal was determined each week by the weight of that animal at the time of administration.

6.5.4 Treatment Regime

Animals were administered COMPOUND XXX by gavage, then 3 hours later L-DOPA was injected IP and the rats then placed immediately into the rotometer test chamber for the next 3 hours. COMPOUND XXX was administered twice a day. On days that the animals were placed in the rotometers, 3 hours later after testing them, the rats were administered their second dose of XXX, followed 3 hours later by L-DOPA.

6.6 Experimental methods

6.6.1 Rotation test

XXX ____, high or low dose, was followed 3 hours later by either a high or low dose of L-DOPA. The rotation test was carried out for the 3 hour time period immediately following L-DOPA/benserazide dose.

6.6.2 Immunohistochemistry

Tyrosine hydroxylase (TH) immunolabeling of the substantia nigra pars compacta (SN-PC) was carried out on all rats. Optical density measurements of the lesioned and non-lesioned SN-PC were then determined since few, if any, remaining TH-labeled neurons were remaining on the lesioned side.

6.7 Body fluids collection: not applicable

6.8 Observations of symptoms

Body weight of the rats was recorded every week.

6.8.1 Method of Sacrifice

The animals were killed by anesthesia with isoflurane, followed by transcardiac perfusion with 300 mls of fixative: 1% acrolein/2% paraformaldehyde in 0.1M phosphate buffer. The brains were removed and placed in phosphate buffer, the *substantia nigra* was cut on a vibratome (70 um sections), and immunolabeling for TH using the diaminobenzidine labelling technique was carried out to determine the loss of TH optical density (ie dopamine cells) on the side of the lesion compared to the non-lesioned side.

6.9 Statistical Analysis

Tests and statistical programs used to analyse the data. One-way ANOVA, making it possible to compare all the groups against each other. When a statistical difference was found, then a post-hoc comparison of multiple means could then be carried out using Tukey-Kramer. A P value of < 0.05 was used to determine statistical significance.

6.10 Archives

All data from this study will be kept on NeuroDetective International's encrypted server. A copy of all raw data from the rotometer and TH-immunolabeling study will be given to Client XXX.

7 Results

7.1 General symptoms observations

Some animals in the high COMPOUND XXX groups (groups 5 and 6) tended to lose weight during the course of the experiment. At the time this became noticeable, extra softened food/water gel was placed in the cages. This appeared to halt the loss of weight.

7.2 Rotation test following L-DOPA in combination with COMPOUND XXX or its vehicle (Appendix 1 – raw data)

Figure 1 shows the mean number of rotations per group per test date over the 3 hour time period that was monitored. The low L-DOPA group showed few rotations during the 4 test days. It did not appear that administration of COMPOUND XXX had any affect on the number of rotations using this low L-DOPA dose. When just the high L-DOPA dose from Groups 4, 5, and 6 were compared against each other, there was no difference between those 3 groups.

Next, the peak time of rotations for each rat at each test date was determined and an overall mean was calculated (**Figure 2**). There was no statistical difference between any of the groups when analyzed together. In addition, when the groups were separated into high and low L-DOPA groups and then compared against each other, there was still no difference between those groups. However, when the individual groups were analyzed separately, there was a difference in peak times for only Groups 5. The peak time of rotations on Day 8 was significantly greater than the peak time for Day 22, ($p=.001$), which was the only significant difference between the 5 testing days.

The mean number of rotations per test day was then analyzed for all the groups during the 180-minute testing period. The number of rotations was calculated every 30 minutes for 3 hours. At the first test day (Day 0, vehicle + COMPOUND XXX only) (**Figure 3**), the animals showed few, if any, rotations during the 3 hour testing period. This data suggest that XXX ____, at either the low or high dose, had no effect on rotations.

Day 1 was the first testing period in which the groups received either a low or high dose of L-DOPA (**Figure 4**). With the low dose L-DOPA, some of the animals rotated a small number of times, but overall there were few rotations. For the high L-DOPA injected groups (4, 5, 6), there was a significant increase in the number of rotations compared to the low L-DOPA treated groups (1,2,3). Comparing just the high L-DOPA treated groups against each other, administration of either the low or high dose COMPOUND XXX resulted in a significant decrease in the number of rotations but only during the first 30 minute time period ($* p < .05$) compared to group 4 (vehicle + L-DOPA). For the rest of the time periods, there was no statistical difference between any of

the high L-DOPA treated groups. At 60 and 120 minutes, there was a trend towards significance (p = .06) in comparing the high dose L-DOPA groups against each other. Further analysis at the 30 minute time period revealed that Group 4 made more rotations than either Group 5 (*p*=.002) or Group 6 (*p*=.011).

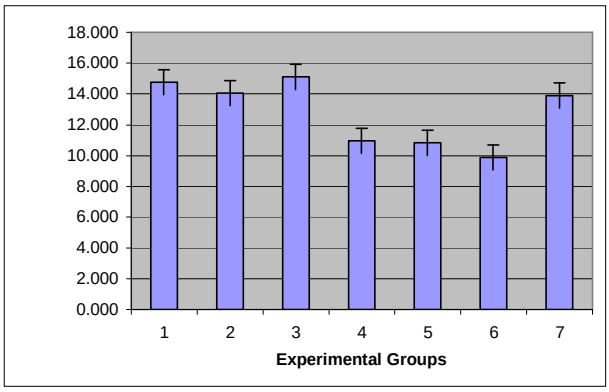
At Day 8 of testing (**Figure 5**), the pattern of rotations was nearly identical to that seen at Day 1. For the high L-DOPA injected groups (4, 5, 6), there was a significant increase in the number of rotations compared to the low L-DOPA treated groups (1, 2, 3). Comparing just the high L-DOPA treated groups against each other, administration of either the low or high dose COMPOUND XXX (i.e., groups 5 and 6) resulted in a significant decrease in the number of rotations but only at the 60 minute time point (* p < .05) compared to group 4 (veh + L-DOPA). For the rest of the time periods, there was no statistical difference between any of the high L-DOPA groups. Further analysis at the 60 minute time period revealed that Group 4 made more rotations than only Group 6 (*p*=.004) and not Group 5, (*p = .16*). There was no difference between Groups 5 and 6 (p = 0.6). The data suggests that after 8 days of L-DOPA treatment, the significant differences in terms of rotations between just the high L-DOPA groups was shifted by 30 minutes to the 60 minute time period.

For test Days 15, 22 and 23, there was no difference between the three high dose L-DOPA groups (**Figures 6, 7, and 8**). However, the statistical analysis at Day 23 of testing revealed a slight trend towards significance (P = .09). The number of rotations for Group 7, which was given a high L-DOPA dose only at this one time period, was similar to that seen for Group 4 at Day 1 [compare Group 4 at Day 1 (Fig. 4) to Group 7 at Day 23 (Fig 8)].

7.3 TH immunohistochemistry (Appendix 1 – raw data)

There was between an 85% and 90% loss of TH immunolabeling of the lesioned compared to the non-lesioned side (see graph below). The full raw data for each of the treatment groups are included in the file named: COMPANY Optical Density.

Effect of a 6-OHDA lesion on the relative optical density of TH immunolabeling within the substantia nigra pars compacta.



There was a significant loss of TH immunolabeling on the lesioned vs the non-lesioned side, ranging between an 85-90% decrease, for all groups (see Table on Page 10 for specific group numbers). All lesioned rats showed a significant loss of TH immunolabeling on the lesioned side. Therefore, no rat needed to be eliminated from the rotational analysis.

8 Discussion and Conclusion

Below is a brief summary of the primary questions that were to be answered as part of the study design:

1.) Does COMPOUND XXX on its own produce rotations in hemiparkinsonian rats? (administration before the first L-DOPA injection)? *No. Using either the low or high dose of XXX ____, the drug had no effect on the number of rotations.*

2.) Does COMPOUND XXX acutely decrease/increase the effect of L-DOPA given at the dose producing rotations? (co-administration with the first high L-DOPA dose after the lesion)? *Yes, but to a small extent. After the first high L-DOPA dose, there is a modest decrease in the number of rotations during the first 30 minutes only, with a trend at the 1 and 2 hour time points*.

3.) Does COMPOUND XXX acutely increase the effect of L-DOPA given at a dose producing no rotations? (co-administration with the first low L-DOPA dose after the lesion)? *No*.

4.) Can the possible add-on potential of COMPOUND XXX be maintained with chronic co-treatment with low-dose L-DOPA? (repeated co-administration with L-DOPA after the lesion)? *No, COMPOUND XXX had relatively little effect when administered with the low L-DOPA dose.*

5.) Is COMPOUND XXX able to reverse or prevent the behavioural consequences of chronic L-DOPA treatment (e.g. exaggeration and

shortening of rotational responses, a putative correlate of wearing-off)? Can it prolong the effects of L-DOPA? *No. In this study, there was no statistically significant shortening of the L-DOPA induced rotational response over the 5 time points that were analyzed.*

Given that COMPOUND XXX is an mGluR5 antagonist, there have been numerous reports using various animal models of Parkinson's disease (PD) that such antagonists may have definite therapeutic potential in this disease [see review by Marino & Conn, Curr Opin Pharmacol 6: 98-102 (2006)]. It has been suggested that in animal models of PD, there is an overactivity of several glutamate pathways, including that from the subthalamic nucleus to the substantia nigra pars compacta and from the motor cortex to the striatum. In the rodent model, we have reported a time dependent change in glutamate activity, presumably originating from the motor cortex [Meshul *et al.*, Neuroscience 88: 1-16 (1999)]. We initially see an increase in extracellular striatal glutamate levels as measured by *in vivo* microdialysis up to one month following the lesion, then a decrease at the 3-month time period. This is correlated with changes in glutamate in the substantia nigra pars reticulata (SN-PR), presumably originating from the subthalamic nucleus (STN), in which we see initially a decrease followed by an increase in extracellular glutamate [Touchon *et al.*, Exp Neurol 193: 131-140 (2005)]. These changes in STN glutamate exactly parallel the changes in striatal glutamate, in that when we observe at 1 month an increase in striatal glutamate following a nigrostriatal lesion, we observe a decrease in glutamate levels in the SN-PR. We have also reported that the apomorphine-induced rotations following a 6-OHDA lesion of the nigrostriatal pathway can be significantly reduced when the motor cortex is subsequently lesioned [Meshul *et al.*, Exp Neurol, 165: 191-206 (2000)]. This suggests the importance of the glutamatergic corticostriatal pathway in terms of the dopamine agonist or even L-DOPA induced rotations in nigrostriatal lesioned animals.

It has been reported by several groups that systemic administration of mGluR5 antagonists reduce the L-DOPA-induced dyskinesias in either the unilateral 6-OHDA lesioned rodent (Armentero *et al.*, Neurobiol Dis 22: 1-9, 2006; Mela *et al.*, J Neurochem 101: 483-497, 2007; Yamamoto & Soghomonian, Neuroscience 163: 1171-1180, 2009; Rylander *et al.*, J Pharm Exp Ther 330: 227-235, 2009) or in the MPTP lesioned monkey (Morin et *al.*, Neuropharmacology 58: 981-986, 2010). Of particular interest from this same group using the MPTP monkey PD model, they recently reported that in both this model and in humans with PD, there was an increase in mGluR5 binding sites in the striatum (Ouattara *et al.*, Neurobiol Aging, in press, 2010). They suggest that in PD, there is an elevation of glutamatergic transmission based on the increase in mGluR5 binding sites. However, based on the in vivo microdialysis studies carried out in our lab (Meshul *et al.*, 1999; Touchon *et al.*, 2005), our data actually suggest a decrease in striatal glutamate transmission. With a decrease in extracellular striatal glutamate levels, this would lead to an increase or sensitization of postsynaptic glutamate receptors and the observed increase in mGluR5 binding sites. However, with systemic administration of mGluR5 antagonists, it is unclear where these drugs may be

acting. A recent study suggests that intracerebral administration of an mGluR5 antagonist into the subthalamic nucleus decreases the motor affects of a 6-OHDA lesion (Phillips *et al.*, Eur J Neurosci 23: 151-160, 2006). However, although mGluR5 antagonists appear to ameliorate the L-DOPA-induced dyskinesias, could this drug be neuroprotective in terms of loss of TH labelled neurons in the SN-PC? It appears that prior treatment with such an antagonist did not modify the loss of TH-labeled neurons in the SN-PC following intrastriatal administration of 6-OHDA (Ambrosi *et al.*, Brain Res Bull, in press, 2010). The drug appeared to give symptomatic relief in terms of the akinesia brought upon by the depletion of striatal dopamine.

COMPOUND XXX reduced the number of L-DOPA induced contralateral rotations at the first two test dates of 1 and 8 days but only during the first 30 minute period or the second 30 minute time period (i.e., at 60 minutes), respectively. After that time, at test days 15, 22, and 23, COMPOUND XXX did not alter the number of L-DOPA induced rotations. Since the L-DOPA in the current study was given at a dose as high as 25 mg/kg twice daily, the drug might have achieved a too-high concentration and have produced a too-high number of contralateral rotations for the effect of XXX -____ to take place. It is also possible that the animals became tolerant to this effect of XXX ____.

It has also been shown that a lesion of the STN prior to administration of 6-OHDA results in a decrease in the loss of TH-labeled cells in the SN-PC (Paul *et al.*, Exp Neurol,185:272-80, 2004). Since COMPOUND XXX presumably blocks postsynaptic mGluR5 receptors, it is possible that even post-treatment with this drug in a partial dopamine lesion rodent PD model (i.e., in MPTP-lesioned mice) would be effective in terms of restoration of TH labelling in the SN-PC. We have preliminary data showing that 7 days after daily administration of MPTP in mice, resulting in about a 50% decrease in TH labelled neurons, subsequent exposure to either an enriched environment or forced exercise on a treadmill for 1 hr/day, resulted in a 30-40% increase in the number of TH labelled neurons. This was correlated with an improvement in locomotor function. Therefore, if COMPOUND XXX would like to be tested in a partial lesion model of PD after the loss of anywhere from 50-60% of the TH neurons, it is recommended that the chronic MPTP model is used.

Another interesting finding from the current study was that over a 22 day time period, there was no significant effect on the time to peak rotations for any of the high L-DOPA groups. In addition, the animals continued to rotate for the entire 3 hour time period. At the 23-day test period, the number of rotations of the chronically treated animals was higher compared to the acute treated group. The time course of the rotations at this point for the chronic versus acute L-DOPA treated groups was nearly the same. This is in contrast to previous work, where it was reported that chronic L-DOPA treatment, using the same dose of L-DOPA as used in the current study, lead to a significant decrease in the duration of the rotations (Engber et al 1994; Karcz-Kubicha et al 1998; Papa et al 1994). However, there is an interesting difference

between those studies and the current study. Following the 6-OHDA lesion, in order to test for the success of the lesion, the other groups used a low dose of apomorphine. This drug results in sensitizing the animals to a subsequent dose of either apomorphine or L-DOPA in terms of the number of rotations, even 2-3 months later (Meshul et al 2002). In the current study, a small dose of amphetamine was used to determine the success of the lesion. Amphetamine does not sensitize the animals to a subsequent dose of L-DOPA as apomorphine does. It is possible that the use of apomorphine in the other studies resulted in a subsequent decrease in the duration of the response to chronic L-DOPA treatment.

The overall summary of the study is:

1.) Overall mean rotation rates for the 3 hours of testing of the high dose L-DOPA over the 5 test days did not differ from each other.
2.) The low L-DOPA treated groups showed very few rotations and administration of COMPOUND XXX at either dose showed little effect on rotations numbers.
3.) When the individual days are analyzed over the 3 hour time period of testing, some differences are seen at the earlier test days (ie days 1 and 8) versus the later test days (i.e., days 15 and 22/23). For test days 1 and 8, there was a statistical difference between the low- and high-dose COMPOUND XXX+ high L-DOPA groups compared to the vehicle + high L-dopa at either the 30 or 60 minute time point, respectively.
4.) There was no difference in the times for the animals to peak in terms of rotations when all groups were compared to each other. However, when the individual groups were analyzed separately, there was a difference in peak times for only the high L-DOPA + low COMPOUND XXX group. The differences in peak times occurred only between Days 8 and 22.

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9 *Figures*

Figure 1:

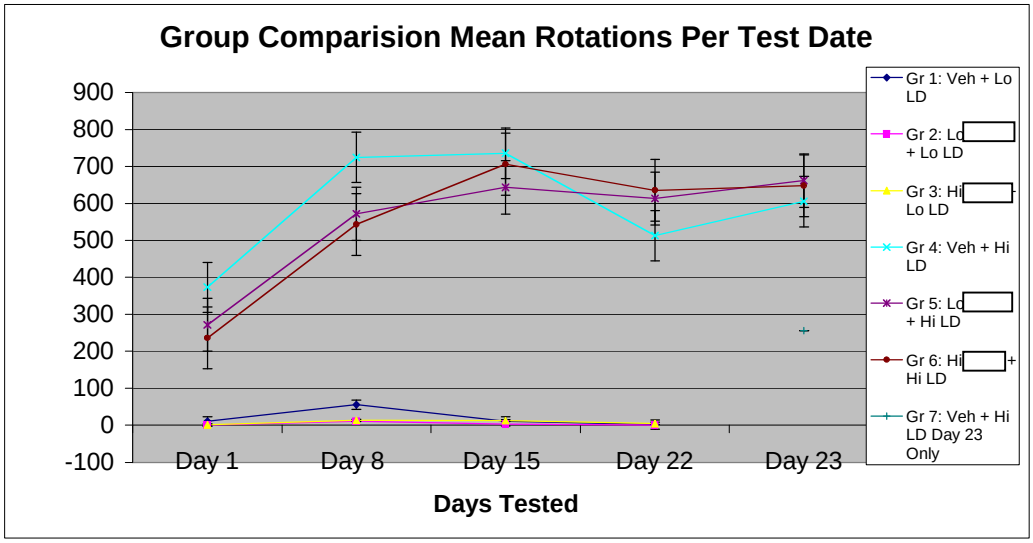

Figure 1: The mean number of rotations during the 3-hour testing period on Days 1, 8, 15, 22 and 23 are shown for each group. In the low L-DOPA treated groups (1, 2 and 3), there were very few rotations. Those values are shown at the bottom of the graph. The high-L-DOPA treated groups showed a significant number of rotations that increased compared to Day 1. However, comparing all the high L-DOPA groups against each other over the 5 test days, the ANOVA showed there was no significant difference between the groups. However, when the individual groups were analyzed separately, there was a difference in Groups 4, 5 and 6 at the various testing days. For Group 4, there was a difference in the mean number of rotations at Day 1 compared to either Day 8 ($p = .001$) and day 15 ($p = .003$). To correct (ie Bonferonni correction factor) for the number of tests run (ie, N = 10), which is 5 test days and comparing 2 different time periods against each other), the p value for significance needs to be reduced from 0.05 to 0.005 (ie .05/10 = .005). For Group 4, there was a trend towards significance in comparing days 1 and 22 ($p = .02$) and comparing days 1 and 23 ($p = .009$). There was also a trend comparing Days 15 and 23 ($p = 0.03$). However, without correcting for the number of tests run, for group 4, Day 1 would have been significantly different compared to all other days. For Groups 5, and 6, there was a difference in the mean number of rotations for day 1 compared to all the other days ($p < .005$). For only Group 6, there was a trend towards a difference between Days 8 and Day 15 ($p = .045$).

Figure 2:

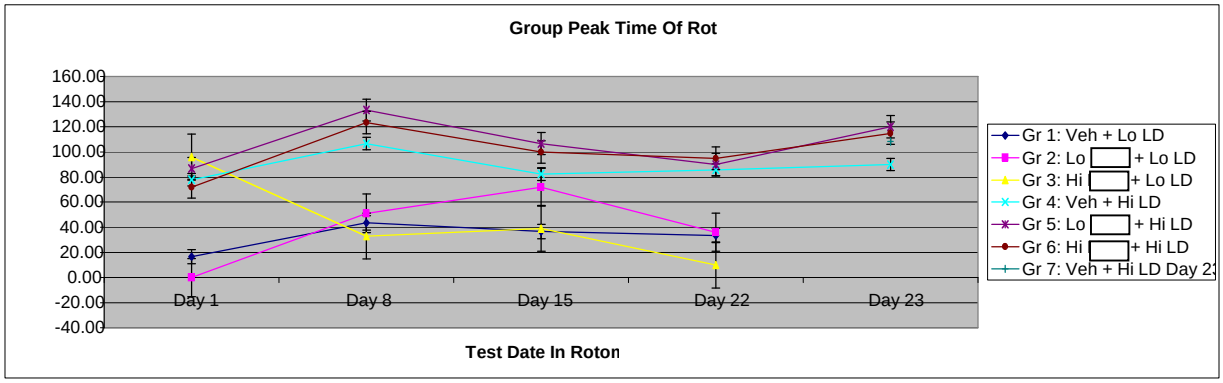

Figure 2: The peak time of rotations for each rat at each test date was determined and an overall mean was calculated. There was no difference in the overall ANOVA analysis. When the groups were separated into high and low L-DOPA groups and then compared against each other, there was still no difference between those groups. For Group 7, given the single injection of high L-DOPA on Day 23, the mean peak rotation time was nearly identical to that of Groups 5 and 6. However, when the individual groups were analyzed separately, there was a difference in peak times only for group 5 ($p = .02$). After a Bonferonni correction for number of tests run (.05/10=.005), the peak time of rotations on Day 8 was significantly greater than the peak time for Day 22, ($p=.001$). This was the only significant result in comparing all the other testing days (see Group Peak Time tab for raw data).

Figure 3: **Mean Rotations by test date: Day 0**

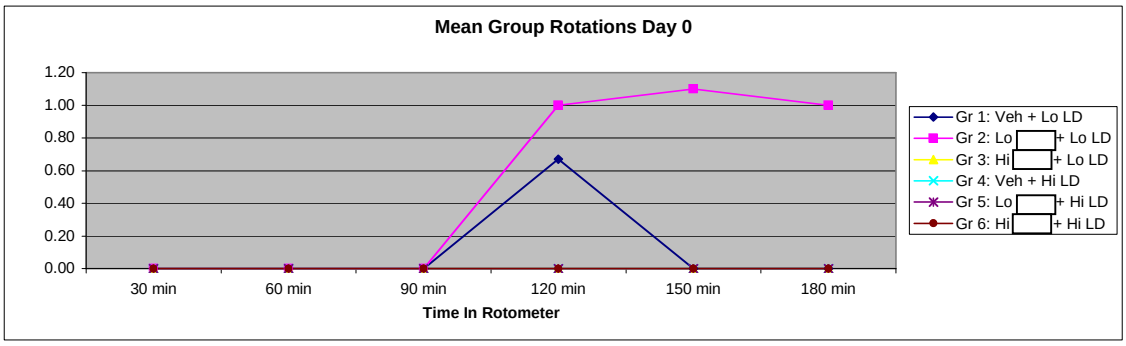

Figure 3: The mean number of rotations on Day 0 when the rats were injected with vehicle + high or low dose of XXX ____. COMPOUND XXX alone resulted in few, if any rotations, for any of the groups.

Figure 4: Mean Rotations by test date: Day 1

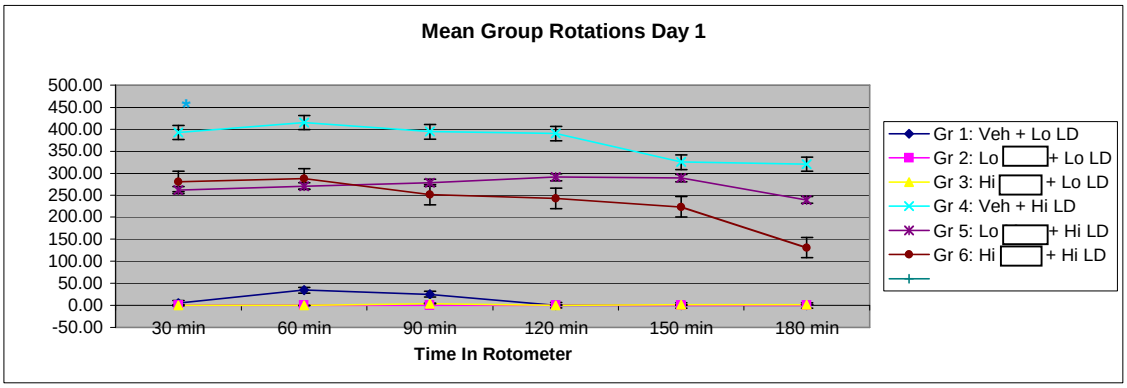

Figure 4: The mean number of rotations on Day 1 for all groups when the rats were injected with the first dose of L-DOPA. For the high L-DOPA injected groups (4, 5, 6), there was a significant increase in the number of rotations compared to the low L-DOPA treated groups (1,2,3). Comparing just the high L-DOPA treated groups against each other, administration of either the low or high dose COMPOUND XXX resulted in a significant decrease in the number of rotations but only during the first 30 minute time period (* $p < .05$) compared to group 4 (veh + L-DOPA). For the rest of the time periods, there was no statistical difference between any of the high L-DOPA groups. However, at 60 and 120 minutes, there was a trend towards significance ($p = .06$). Further analysis at the 30 minute time period revealed that Group 4 made more rotations than Group 5 (p=.002) and Group 6, (p=.011). (see tab: Mean Rotations by Date and Day 1 Mean Rotation by Time for details).

Figure 5: Mean Rotations by test date: Day 8

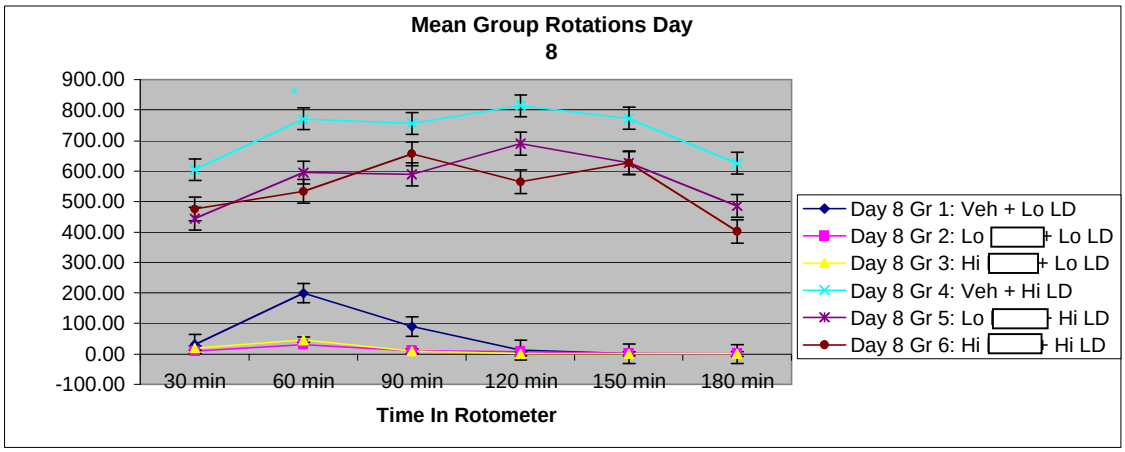

Figure 5: The mean number of rotations on Day 8 for all groups when the rats were injected with L-DOPA. For the high L-DOPA injected groups (4, 5, 6), there was a significant increase in the number of rotations compared to the low L-DOPA treated groups (1,2,3). Comparing just the high L-DOPA treated groups against each other, administration of either the low or high dose COMPOUND XXX (ie groups 5 and 6) resulted in a significant decrease in the number of rotations but only at the 60 minute time point (* $p < .05$) compared to group 4 (veh + L-DOPA). For the rest of the time periods, there was no statistical difference between any of the high L-DOPA groups. Further analysis at the 60 minute time period revealed that Group 4 made more rotations than only Group 6 (p=.004) and not Group 5, ($p = .16$). There was no difference between Groups 5 and 6 ($p = 0.6$) (see tab: Mean Rotations by Date and Day 8 Mean Rotation by Time for details).

Figure 6: Mean Rotations by test date: Day 15

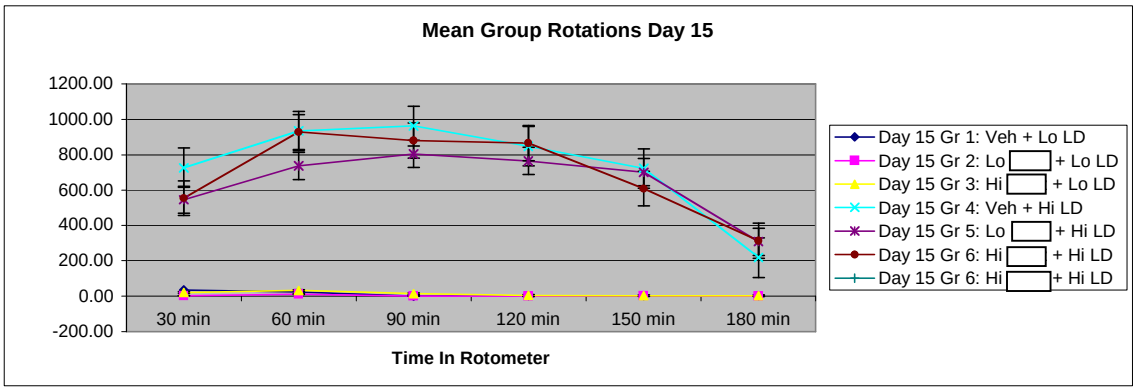

Figure 6: The mean number of rotations on Day 15 for all groups when the rats were injected with L-DOPA. For the high L-DOPA injected groups (4, 5, 6), there was a significant increase in the number of rotations compared to the low L-DOPA treated groups (1,2,3). Comparing just the high L-DOPA treated groups against each other, administration of either the low or high dose COMPOUND XXX resulted in no significant difference compared to the L-DOPA only group (4) at any of the time periods. (see tab: Mean Rotations by Date and Mean Rotations HI LD vs LO LD for details).

Figure 7: Mean Rotations by test date: Day 22

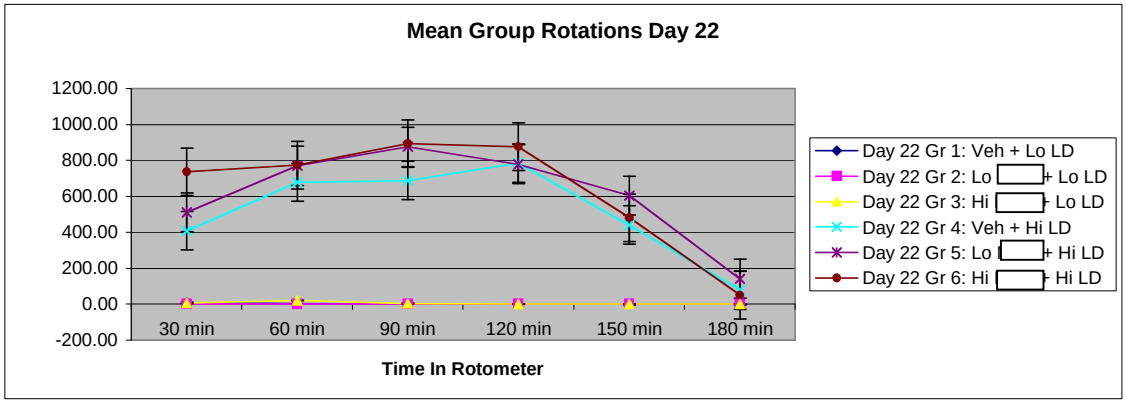

Figure 7: The mean number of rotations on Day 22 for all groups when the rats were injected with L-DOPA. For the high L-DOPA injected groups (4, 5, 6), there was a significant increase in the number of rotations compared to the low L-DOPA treated groups (1,2,3). Comparing just the high L-DOPA treated groups against each other, administration of either the low or high dose COMPOUND XXX resulted in no significant difference compared to the L-DOPA only group (4) at any of the time periods. (see tab: Mean Rotations by Date and Mean Rotations HI LD vs LO LD for details).

Figure 8: Mean Rotations by test date: Day 23

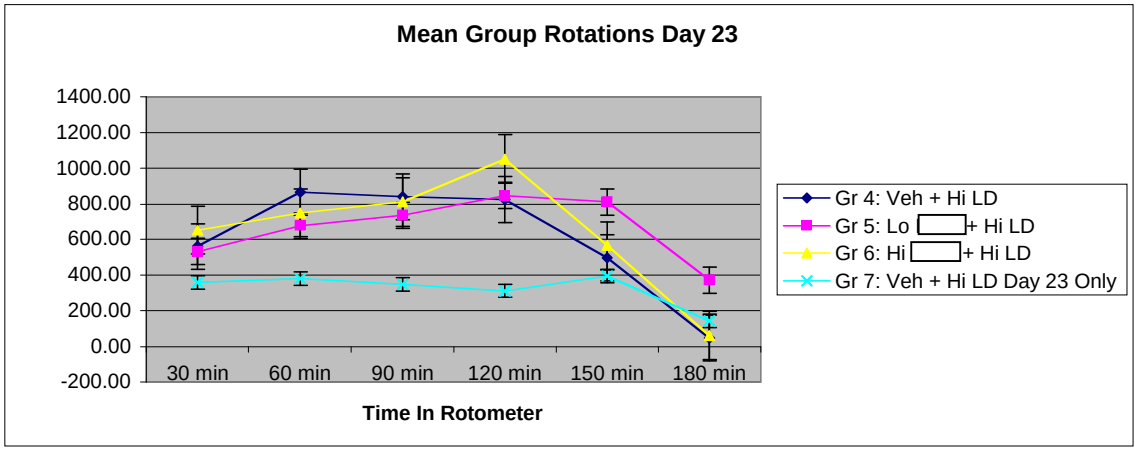

Figure 8: The mean number of rotations on Day 23 for groups 4-7 when the rats were injected with L-DOPA. For Group 7, this was the first and only injection of the high L-DOPA dose. The ANOVA revealed no significant difference between any of the groups (P = 0.09) (see tab: Mean Rotations by Date for details).

10 Tables

10.1 Table 1

See attached spreadsheet for all raw data.

11 Appendices

11.1 Appendix 1

See attached spreadsheet of all raw data and all analysis.

11.2 Appendix 2

11.3 Appendix 3